KALANCHOE PINNATA PREPARATION IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS

A Thesis

by

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

The aqueous preparation of *Kalanchoe pinnata* is traditionally used in the management of Type 2 diabetes mellitus (T2DM), but the effectiveness in curtailing the indices of T2DM is not clear. In this study, the efficacy of *K. pinnata* preparation in the treatment of T2DM animal models was investigated.

Forty-eight Sprague-Dawley rats were divided into eight groups of six rats each as follows: Group 1: non-diabetic rats fed normal rat diet, group 2: non-diabetic rats fed high fat diet (HFD), group 3: Type 2 diabetic rats fed HFD, group 4: non-diabetic control group fed normal diet, group 5: non-diabetic control group fed HFD, group 6: diabetic control group, group 7: diabetic plus *K. pinnata* preparation, and group 8: diabetic plus Metformin. Groups 1 – 3 were euthanized after 21 days to generate baseline data. Thereafter, *K. pinnata* preparation or metformin was administered for 28 days. Animals were euthanized on day 28 after an overnight fast. Blood and organs were collected for various assays.

Type 2 diabetic rats treated with *K. pinnata* preparation lost significant ($P < 0.05$) weight. *Kalanchoe pinnata* consumption resulted in decreased serum glucose. There was a significant ($P < 0.05$) increase in HDL and decrease in triglyceride levels. Alkaline phosphatase (ALP) and alanine amino transferase (ALT) activities, and blood urea nitrogen (BUN) and uric acid (UA) levels were significantly ($P < 0.05$) increased. Serum reduced glutathione (GSH) levels, superoxide dismutase (SOD) and hepatic pyruvate kinase (PK) activities were significantly ($P < 0.05$) elevated.

Overall, results show that the consumption of *K. pinnata* preparation in Type 2 diabetic rats decreased body weight and serum glucose levels, as well as increased serum HDL and decreased triglyceride levels which may be beneficial in the effective management of the disease. Similarly, the observed increase in SOD activity and GSH levels in the diabetic rats may be protective against oxidative stress. The observed increase in hepatic PK activity may be indicative of improved glucose metabolism. However, the increase in serum ALP and ALT activities, and BUN and UA levels may be suggestive of hepatic and renal damage.

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1. Introduction

Background and Relevance

1.1 Epidemiology of diabetes mellitus

Type 2 diabetes is or can be a slow deteriorating disease if the proper treatment is not sought. Natural remedies for this disease have been pursued to help alleviate the expense and associated aftereffects of modern treatment. Type 2 diabetes is more widespread than Type 1 diabetes. The World Health Organization (WHO) estimates that it accounts for 90% of all diabetes worldwide. Obesity and lack of physical activity are associated with the development of Type 2 diabetes. Within the next 10 years, total deaths are estimated to increase to more than 50% due to diabetes. Once a rare occurrence, children are now being affected by Type 2 diabetes. Half of the newly reported cases in some countries are children and teenagers. In 2013 WHO reported that approximately 347 million people worldwide have diabetes. Type 1 diabetes is caused by the lack of insulin; it cannot be prevented and is treated with injections of insulin. Gestational diabetes is developed during pregnancy and these women are at risk of developing Type 2 diabetes later in life. In 2012, an estimated 2.2 million people died from high blood glucose levels [1]. Diabetes is projected to become the seventh leading cause of death in the world by 2030. About 50% to 80% of deaths attributed to diabetes is due to cardiovascular disease (CVD). Eighty percent of deaths due to diabetes take place in middle and low economic countries. However, in areas that are economically developed, most individuals with diabetes are within retirement age versus developing areas where those diagnosed with the disease are younger (between the

ages of 35 and 64). Primary complications associated with diabetes are amputation, kidney failure and blindness. Individuals are prone to complications associated with diabetes due to the general public's lack of knowledge, costly medications and lack of health care amenities. Type 2 diabetes can be prevented by adopting a healthy lifestyle [1]. The CDC (Centers for Disease Control and Prevention) and other organizations have put together prevention programs to educate the public and those that have an increased risk of acquiring the disease. This includes group support, dietary education and at least 150 minutes per week of physical activity. Prevention is primarily directed towards those more susceptible, in order to reduce its prevalence. Factors that increase the chances of developing diabetes include an inactive lifestyle, older age, family history of diabetes, gestational diabetes, and ethnicity. Ethnic groups that have a higher risk for developing Type 2 diabetes are Hispanics, African-Americans, Asian-Americans, Pacific Islanders, American Indians and Alaska Natives. The CDC reported that 1 in every 3 U.S. adults could develop diabetes by 2050, if current trends continue. These estimations are due to longer life expectancies and the increase in the population of minorities, those that have a higher risk in developing Type 2 diabetes. Presently, 1 in every 11 U. S. adults has diabetes. The International Diabetes Federation reported that 285 million people worldwide had diabetes in 2010. By 2030, they predict that 438 million will have diabetes. In the U.S. the expenditures associated with having diabetes are an estimated $174 billion annually. Six million Americans has diabetes and do not know they have it [2]. The CDC determined in 2012 that 37% or 86 million U.S. adults, 20 years or older had pre-diabetes. Pre-diabetes occurs when fasting blood sugar levels are higher than normal but not as high to be considered diabetic, between 100 mg/dL to 125 mg/dL. Conditions associated with

diabetes are hypoglycemia, hyperglycemia, high blood pressure, high blood LDL (low density lipoproteins) cholesterol, heart disease, stroke, kidney failure, blindness, as well as amputation of the lower-limbs. Between 2003-2006 the death rates for cardiovascular disease were higher for those 18 years and older with diabetes than for those without diabetes. Heart attack and stroke rates were higher for those 20 years or older with diabetes in 2010. Between 2005-2008, 28.5% of those 40 years or older in the U.S., had diabetic retinopathy, micro-vascular damage to the retina of the eye that may lead to loss of vision. In 2011, diabetes was the leading cause of kidney failure in 44% of diabetic individuals. In 2011, 49,677 individuals of all ages were treated for kidney failure due to diabetes, many with chronic dialysis or kidney transplant. Nearly 60% of individuals with diabetes endure lower-limb amputations. Micro-vascular complications can be avoided by maintaining normal blood glucose levels. Complications of Type 2 diabetes can potentially be avoided by utilizing early detection methods, dilated eye exams, foot exams, monitoring blood pressure, urine and blood tests, [3]. Current estimations and predictions point to the importance of identifying effective treatment of the disease. Natural remedies, such as the use of medicinal plants may be a safer alternative for the treatment of the disease compared to conventional treatment with drugs. The problem with medicinal plant alternatives is lack of scientific research and data to support its use.

1.2 Type 2 diabetes mellitus

Diabetes Mellitus is the hyperglycemic state of an individual due to insulin resistance (Type 2 diabetes) or lack of insulin produced (Type 1 diabetes) by the pancreas. Due to the absence or resistance of insulin, cells are not able to take up glucose for energy generation.

The pancreas contains an endocrine-exocrine system. The islets of Langerhans (endocrine) beta cells release insulin and the acinar cells (exocrine) use ducts to release digestive enzymes, such as amylase, into the duodenum. Yadav et al. [4] reported that significantly low levels of serum amylase in Type 2 diabetic patients are associated with compromised insulin action. Continuous damage to the acinar cells is observed during insulin shortage. In Type 2 diabetes, insulin is unable to efficiently attach to the cells insulin receptor tyrosine kinase (IR) in order to initiate a protein phosphorylation cascade and allow the entrance of glucose into the cell for metabolism [5]. The pancreas then produces more insulin to overcome insulin resistance, but over time the beta-cells malfunction and become damaged. Leptin is a hormone that is released from adipose tissue. It suppresses food intake and induces weight loss [6]. Fischer et al. [7] reported that Type 2 diabetic patients with the most insulin resistance had the highest fasting leptin levels. Leptin levels are found to be increased in those that are obese, Type 2 diabetics that are obese tend to show reduced levels of leptin, they suspect it may be caused by altered fat distribution. It was also reported that those that are Type 2 diabetic and lean show reduced levels of leptin [8]. Mohammadzadeh et al. [9] reported that low serum leptin levels in diabetic patients with a relatively damaged pancreas exhibited insulin resistance, lower beta cell function and consequently metabolic abnormalities. Varying results from different studies have been found for Type 2 diabetic patients and leptin levels. This may be because it is affected by many variables such as body mass index, gender, adiposity, insulin levels and insulin sensitivity [9]. Wauters et al. [10] reported that a significant factor in leptin production in Type 2 diabetic patients was the secretion of insulin and the level of insulin resistance. To compensate for insulin resistance in non-diabetic patients the adaptation of appropriate

insulin secretion occurs, this is not the case for Type 2 diabetic patients, the adaptation is not sufficient. Islet malfunction plays a crucial role in the progression of insulin resistance in Type 2 diabetic patients [11]. There are genetic and environmental factors associated with the onset of Type 2 diabetes. Individuals with a family history of Type 2 diabetes have a 40% chance of developing the disease. Obesity is also a key player in the development of Type 2 diabetes. Peripheral insulin resistance occurs when free fatty acids escape from adipose cells, the liver takes up the free fatty acids and causes fasting hyperglycemia. Symptoms of Type 2 diabetes are polyuria, polyphagia, increased appetite and polydipsia [12]. Polydipsia is excess thirst due to the increased blood glucose levels and elevated osmotic pressure. This eventually prevents water from being absorbed back into the bloodstream and is then excreted in excess, which is known as polyuria [13]. Hyperglycemia may cause neuropathy due to excess sugar in the capillaries that nourish nervous tissue, it decreases neurovascular flow. Studies of streptozotocin-induced rats have found nerve demyelination, endoneural edema and axonal atrophy associated with Type 1 and Type 2 diabetes [14]. Consistent hyperglycemia causes an increase in polyol pathway activity due to the build-up of sugars in nerves, this damage is caused by an unknown mechanism. Nerve degeneration is also caused by decreased myo-inositol absorption and inhibition of the sodium/potassium ATP pump which results in sodium retention [15]. This causes tingling, numbness, and burning sensations, complete loss of sensation is possible in affected limbs. The lack of sensation to the limbs can cause nerve damage and lack of blood circulation to the feet can result in foot ulcers, which can become infected and lead to amputations. Type 2 diabetes may be difficult to detect because individuals may be asymptomatic. To diagnose diabetes, patients require thorough analysis of testing including

clinical history, fasting and random plasma glucose levels, glycated hemoglobin testing (HbA1c) and oral glucose tolerance testing (OGTT). Random glucose levels greater than 200 mg/dL are indicative of diabetes. If fasting levels are ≥ 126 mg/dL or more diabetes is confirmed. OGTT is considered a high quality test in the measurement of glucose levels. Seventy-five grams of glucose is consumed and blood glucose levels are measured two hours later. OGTT is used to test how well a patient is able to metabolize a dose of glucose, having fasted overnight. HbA1c testing is also used to diagnose diabetes. It provides the patients' blood glucose levels from erythrocytes over a 3-month time span and can show how well glucose levels have been maintained. To treat and manage Type 2 diabetes a healthy diet, active lifestyle, and medication are recommended. The key to the management of overweight Type 2 diabetics is weight loss, just by losing 5-10% body weight can help with glucose control. If Type 2 diabetes is not detected early enough and treated with non-medicinal methods such as exercise and dieting, using oral hypoglycemic medication to treat it may be necessary because it is a progressive disease. Metformin is frequently used as a treatment for Type 2 diabetes, but side effects include nausea and abdominal cramps. Type 2 diabetic medication causes the risk of hypoglycemia; it is important to monitor blood glucose levels [16]. Hyperglycemia over a long period of time can result in advanced glycation end products, advanced oxidation protein products, and low-density lipoprotein accumulation. These are associated with diabetes and cardiovascular disease where cell damage occurs due to oxidative stress. Type 2 diabetes characterized by hyperglycemia can affect plasma proteins such as albumin, globulin and total proteins through glycation. Glycation is a non-enzymatic process in which covalent bonds form between glucose and plasma proteins. In Type 2 diabetics glycation of plasma proteins was reported to lower

non-covalent interaction affinities for polyphenols, which play a role in removing free radicals. Insulin deficiency is known to lower albumin levels because insulin controls albumin gene expression [17]. Chen et al. [18] reported that with the signaling of insulin disrupted in livers by the removal of the insulin receptor gene, *Akt*, albumin expression was significantly decreased. Low-density lipoprotein accumulation has adverse effects due to the body's ability to transport fat molecules into artery walls, causing inflammation and ultimately cardiovascular disease [12]. Lipid metabolic abnormalities are associated with Type 2 diabetes and cardiovascular disease (CVD). Insulin plays a major role in the metabolism of lipoproteins, which allow fats to be carried within the blood stream. Hyperglycemia, obesity and insulin action associated with Type 2 diabetes quicken the advancement of atherosclerosis. Ozder [19] reported that Type 2 diabetic patients had higher levels of cholesterol, triglycerides and lower HDL. Fang et al. [20] reported that serum levels of phosphorus were reduced in Type 2 diabetic patients, and suggested that there may be a malfunction in phosphorus metabolism. They also reported that there was no correlation between Type 2 diabetes and levels of serum calcium [20]. Glucose is more effective than inorganic phosphate in accelerating the uptake of sodium, during hyperglycemia, depolarization of the microvilli covered membrane allows the entrance of inorganic phosphate into the tubular cells of the kidneys. This reduces intracellular phosphate, which causes hyperphosphaturia, excess concentration of phosphates in the urine [21]. Elevated white blood cell counts (WBC) are associated with impaired glucose tolerance test. Micro- and macroangiopathy are both linked to high WBC levels in Type 2 diabetes [22]. Microvascular complications have been associated with a decreased red blood cell count (RBC) in Type 2 diabetic patients [23]. Deteriorating glomerular filtration

rate in kidney disease is associated with low hemoglobin (HGB) levels in diabetic patients. Patients that have diabetic nephropathy and retinopathy are increasingly vulnerable to a reduction in HGB levels. The mechanism is unknown but some suggest that beta cell disruption arises due to a lack of oxygen to the islet cells of the pancreas [24]. Vascular injury in Type 2 diabetic patients can be due to insulin resistance, inflammation and oxidative stress [12]. The beta cells in the pancreas produce insulin to facilitate glycolytic metabolism due to increased levels of blood glucose. Reactive oxygen species (ROS) such as hydrogen peroxide have been documented to function as a cellular signal transductor and has the capacity to adjust glucose-stimulated insulin secretion in beta cells [25]. In the past, ROS were considered waste but this mitochondrial byproduct is necessary for biological reactions. Cellular respiration produces superoxide radicals which are produced as a byproduct, it is highly reactive and can grab electrons from other molecules. Superoxide can potentially be converted to the less harmful, hydrogen peroxide by superoxide dismutase (SOD) and then converted to oxygen and water by catalase (CAT). The beta-cells in the pancreas are able to convert superoxide into hydrogen peroxide within the mitochondria and cytoplasm [25]. There is a low concentration of CAT to inactivate the hydrogen peroxide within the beta-cells. This imbalance of SOD and CAT allows the accumulation of hydrogen peroxide. Hydrogen peroxide is a stable, small, uncharged molecule, and can diffuse freely. It can be produced and destroyed quickly in response to external stimuli and is considered an intracellular messenger. In large concentrations ROS or hydrogen peroxide can be destructive to cells [25]. Oxidative stress has been associated in the pathogenesis of beta-cell failure. ROS derived from glucose metabolism serves as a metabolic signal for insulin secretion. Figure 1 shows a model of reactive oxygen species

as a signal in glucose stimulated insulin secretion and the relationship to oxidative stress. Transcription factor NF-E2-related factor 2 (Nrf2) regulates cellular adaptive responses to oxidative stress in order to protect cells from oxidative damage. By activating this transcription factor with free radicals, it triggers the release of antioxidant enzymes, which destroy the reactive oxygen species and can no longer signal for insulin secretion [25]. Free radicals are unstable reactive oxygen species that can potentially mutate DNA, proteins and lipids. This mutation and damage can cause many diseases. Complications associated with oxidative stress and diabetes are endothelial damage of both macrovascular and microvascular tissues [26]. Insulin secretion is triggered by low levels of hydrogen peroxide, but high levels can be toxic to pancreatic cells [25]. Type 2 diabetic patients showed decreased blood catalase (CAT) concentrations [27]. Rajagopal et al. [28] has established that reduced glutathione (GSH) production is decreased in Type 2 diabetic patients, this ultimately causes tissue damage due to oxidative stress. Zhao et al. [29] reported that reduced activity in superoxide dismutase (SOD) in Type 2 diabetic rats due to hyperglycemia stimulated oxidative damage. Increased levels of thiobarbituric acid reactive substances (TBARS) are associated with Type 2 diabetes. TBARS are used as an indicator for lipid peroxidation, degradation of lipids due to free radicals, which can prove potentially harmful to cell membranes of beta cells in the pancreas [30]. When carbohydrates are available, metabolism in the liver is initiated, the liver uses glucose as its key energy source and converts it into fatty acids. Both liver and kidney have enzymes responsible for glycogenesis. Glucose is hydrolyzed into pyruvate through the process of glycolysis by using malic enzyme (ME) to convert malate into pyruvate and yielding NADPH. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes glucose-6-phosphate to

produce NADPH, it is the first regulatory step of the pentose phosphate pathway. NADPH provides the reducing power for lipogenesis. The rate at which molecules progress through the glycolytic pathway is controlled by pyruvate kinase (PK). Pyruvate kinase usually increases once food has been consumed. Consumed carbohydrates initiate lipogenesis. The key glycolytic product is pyruvate, and it offers a carbon to initiate lipogenesis and this in turn links glycolysis to lipogenesis [31]. In Type 2 diabetic patients it has been shown that there are increased levels of interleukin-6 (IL-6), a pro-inflammatory cytokine. Schultz et al. [32] reported that by inhibiting IL-6, insulin sensitivity is enhanced in patients with compromised immunity, this suggests that high levels of IL-6 in Type 2 diabetic patients may be involved in the pathology of insulin resistance. Figure 2 shows diseases/disorders caused by oxidative stress. Oxidative stress due to free radicals can damage the immune response and increase the potential for cell and protein damage and insulin resistance. Cardiovascular diseases are also associated with oxidative stress in the form of atherosclerosis, hypertension, diabetes and kidney disease. ROS have the potential to cause endothelial dysfunction and inflammation. Endothelial dysfunction is destruction of the interlining of the blood vessels which hinders its ability to constrict and dilate for the passage of blood and other substances [33].

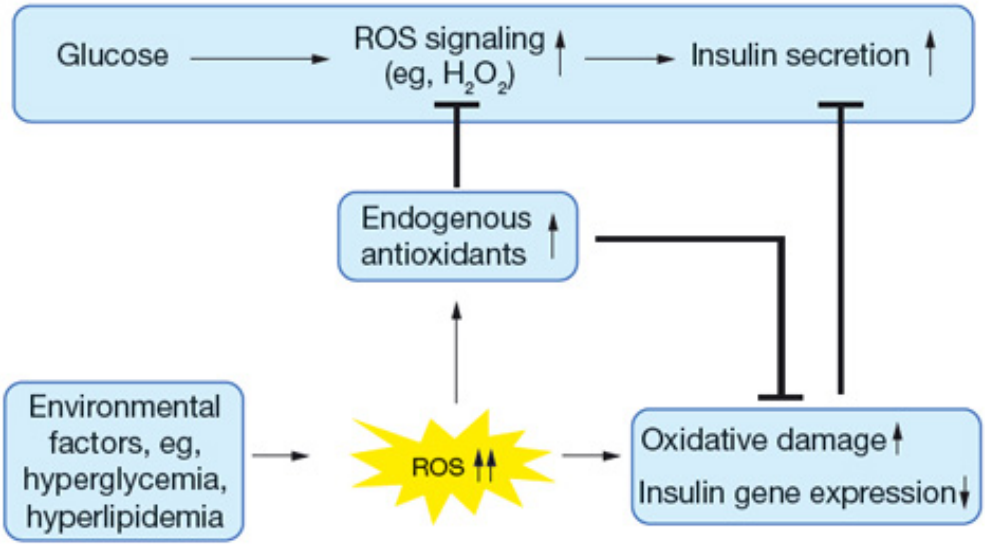

Figure 1. Accumulation of endogenous antioxidants due to excess reactive oxygen species can hinder glucose stimulated insulin secretion. Adapted from Pi [25].

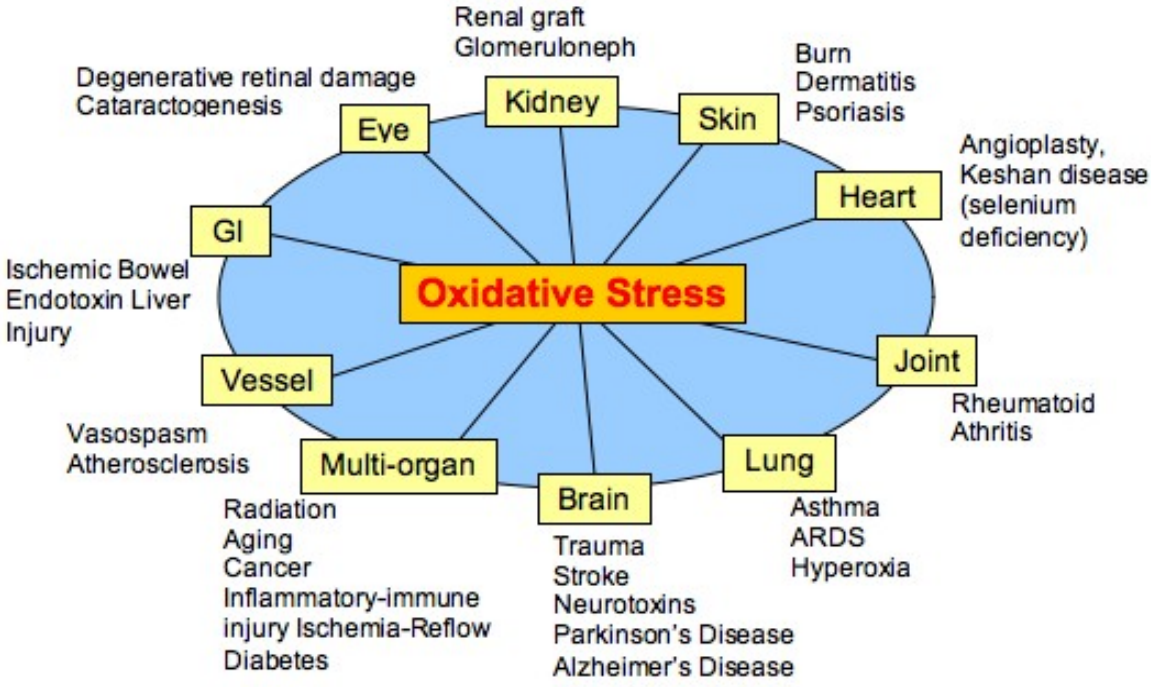

Figure 2. Diseases/disorders caused by oxidative stress. Adapted from Mugesh [33].

1.3 Description of *Kalanchoe pinnata*

Kalanchoe pinnata, is a medicinal plant native to South America, Africa, and the Caribbean. *Kalanchoe pinnata* grows well in tropical, sub-tropical or warmer temperate regions. This plant has a moist, sturdy, tuberous, hairless stem. The leaves are simple and thick with 3-5 lobes. Flowers hang loosely with orange-red coloration. *Kalanchoe pinnata* has the ability to produce plantlets on the superficial edges of its leaves [34].

Figure 3. *Kalanchoe pinnata* plant and its flowers. Adapted from Gardening on cloud 9 [35].

1.4 *Kalanchoe pinnata* in the treatment of diabetes mellitus

This plant exhibits medicinal properties for the treatment of many diseases such as chronic bronchitis, pneumonia, and inflammation, it also has the ability to modulate the effects of the immune system using aqueous preparations of its leaves or stems [34]. Figure 3 shows *K. pinnata* plant and its flowers [35]. The leaves of *K. pinnata* contain a collection of chemicals, which include but are not limited to flavonoids, alkaloids, triterpenes,

glycosides, and steroids [36]. It is believed that these chemicals have anti-inflammatory and anti-diabetic properties. A study conducted by Ojewole showed that administration of the liquefied extract of *Bryphyllum pinnatum* leaf to streptozotocin-induced diabetic rats resulted in the significant reduction in blood glucose levels [36]. Isolated quercetin flavonoid extract from *K. pinnata* fed to mice were shown to deter lethal anaphylaxis in 75% of the tested group. This shows that treatment with *K. pinnata* reduces the immune response [37]. Quercetin was also shown to decrease body weight gain induced by high-fat diet [38, 39]. A preliminary study from our laboratory showed a decrease in body weight, and hypoglycemic and hypocholesterolemic activities in Type 1 diabetic rats administered aqueous preparation of *K. pinnata*. Traditionally, the aqueous preparation of *K. pinnata* is used in the management of Type 2 diabetes mellitus [40]. However, the effectiveness of aqueous preparation of *K. pinnata* in curtailing the indices of Type 2 diabetes is not clear. This study is aimed at determining the effectiveness of *K. pinnata* aqueous preparation in the treatment of Type 2 diabetes in animal models of the disease.

1.5 Objectives

The study:

(1) determined serum glucose levels, lipid composition, renal and liver function enzymes, and intestinal amylase activity in streptozotocin-induced Type 2 diabetic rats and evaluated the effects of the aqueous *K. pinnata* consumption on these biochemical parameters. The goal was to determine whether the consumption of aqueous preparation of *K. pinnata* reduced serum glucose levels, and altered lipid composition as well as intestinal amylase

activity. Kidney and liver function enzymes were investigated to establish beneficial or adverse effects of the consumption of *K. pinnata* on organ integrity.

(2) determined systemic levels of leptin and insulin and established whether *K. pinnata* aqueous preparation consumption regulates appetite and body weight in Type 2 diabetic rats in light of the preliminary report from our lab that indicated a decrease in body weight in streptozotocin-induced Type 1 diabetic rats fed aqueous preparation of *K. pinnata*.

(3) evaluated oxidative stress, inflammatory responses, hepatic and renal lipid and carbohydrate metabolism in streptozotocin-induced Type 2 diabetic rats and established the effectiveness of aqueous *K. pinnata* preparation consumption in the management and prevention of complications associated with Type 2 diabetes.

2. Materials and Methods

2.1 Research Location and Logistics

This study was conducted in two separate locations. The first part, conducted in the vivarium at Texas A&M University-Kingsville, included the induction of Type 2 diabetes in rats using high fat diet and low dose of streptozotocin and the subsequent treatment of the diabetic rats with *Kalanchoe pinnata* aqueous preparation for four weeks' post-induction. The second part of the study was conducted in Dr. Felix Omoruyi's/ Jean Sparks' lab at Texas A&M University-Corpus Christi, Natural Resources Center building, room number 3416. This room provided sufficient tools for all the assays that were performed. The IACUC protocol number was 019249.

2.2 Research Design

Forty-eight Sprague-Dawley rats (24 females and 24 males), weighing 150-200 g were divided into eight groups of six rats each. Thirty-six Sprague-Dawley rats were fed a high-fat diet for three weeks followed by a single injection of streptozotocin (Sigma-Aldrich, 35 mg/Kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally (IP) to induce Type 2 diabetes in 24 of the 36 rats fed high fat diet on day 14 [41, 42]. Twelve non-diabetic rats fed high fat diet and 12 control rats fed normal rat diet were injected IP with an equivalent amount of buffer (0.05 M-citrate buffer, pH 4.5). Seven days after injection, rats with a blood glucose level $\geq$ 240 mg/dL were considered as Type 2 diabetic rats and used for further experiments [43]. Diabetes was identified by pricking the tail for a drop of blood after an overnight fast for glucose determination using a strip-operated blood glucose meter (Bayer Contour Blood Glucose Monitoring System) on day 21 for all animals. The blood glucose of all animals that were injected with streptozotocin intraperitoneally exceeded 240 mg/dL. Previous studies have demonstrated that Type 2 diabetic rodent models can be developed by feeding high-fat diet and administering low dose of streptozotocin [44, 45, 46]. The principle is as follows; the consumption of high fat diet results in the development of insulin resistance, while the administration of a low dose of streptozotocin (35 mg/kg body weight) results in a mild impairment of beta cell function which subsequently leads to hyperglycemia. The method was used to develop the animal model of Type 2 diabetes in this study [43]. Animals were grouped as follows: Group 1: six non-diabetic rats fed normal rat diet (baseline control), group 2: six non-diabetic rats fed high fat diet (baseline high fat diet group) and group 3: six Type 2 diabetic rats fed high fat diet (baseline

diabetic). Groups 1-3 were euthanized by decapitation after an overnight fast for the generation of baseline data before treatment (week zero). After 21 days on high fat diet, the rats were given normal diet with or without treatment for an additional 28 days as follows: Group 4: non-diabetic control group fed normal diet, group 5: non-diabetic control group fed high fat diet, group 6: diabetic control group, group 7: diabetic plus aqueous preparation of *K. pinnata*, and group 8: diabetic plus Metformin, a standard anti-diabetic drug, was used as a positive control. The aqueous preparation of *K. pinnata* (0.14 g/kg body weight/day) or Metformin (300 mg/kg body weight/day) was administered once a day via gavage for 28 days. No insulin was administered and expected clinical signs included polydipsia, polyuria and body weight loss (less than 10%) for the diabetic groups. Changes in blood glucose were assessed weekly by pricking the tail for a drop of blood after an overnight fast. Daily water consumption was recorded. Body weight changes and total food intake were recorded weekly. Animals were euthanized by decapitation on day 28 after the commencement of the feeding trial excluding the 21-day period for the development of the animal model of the disease after an overnight fast. Blood (collected in purple top tubes – for hematological assays and red top tubes – chemistry assays) and organs (kidneys, liver and small intestine) were collected for various assays.

Table 1. Experimental design summary

Groups		IP Injection (Day 14)	*Dietary Supplement* (4 weeks excluding the first 21 days)
Baseline Control	**Normal rat diet**	**Citrate buffer**	**Euthanized on day 21**
Baseline High Fat Diet	**High fat diet**	**Citrate buffer**	**Euthanized on day 21**
Baseline Diabetic	**High fat diet**	**35 mg of Streptozotocin in Citrate buffer/Kg Body Wt.**	**Euthanized on day 21**
Control	**Normal rat diet**	**Citrate buffer**	**Normal rat diet euthanized on day 49**
High Fat Diet	**High fat diet**	**Citrate buffer**	**Normal rat diet euthanized on day 49**
Diabetic Control	**High fat diet**	**35 mg of Streptozotocin in Citrate buffer/Kg Body Wt.**	**Normal rat diet euthanized on day 49**
Diabetic *K. pinnata*	**High fat diet**	**35 mg of Streptozotocin in Citrate buffer/Kg Body Wt.**	**Normal rat diet + *K. pinnata* preparation via gavage euthanized on day 49**
Diabetic Metformin	**High fat diet**	**35 mg of Streptozotocin in Citrate buffer/Kg Body Wt.**	**Normal rat diet + Metformin via gavage euthanized on day 49**

2.3 Objective 1

Preparation of intestinal mucosa homogenate

The intestine of each rat was divided into two portions: the upper intestine (proximal) and lower intestine (distal). Rat intestine, free of food materials, was excised and the lumen flushed out several times with 0.9% NaCl. The mucosa was scraped, and combined with the washed mucosa and then homogenized and centrifuged at 5,000 x g. The supernatant was frozen until required for enzymatic assays [47].

Determination of serum amylase

Serum amylase was measured using Stanbio alpha-amylase assay kit. Amylase hydrolyzes p-nitrophenyl D-maltoheptaoside (PNPG7) to p-nitrophenyl-maltotriose (PNPG3) and maltotetraose. PNPG3 was then hydrolyzed by glucoamylase to p-nitrophenylglycoside (PNPG1) and glucose. Glucosidase hydrolyzes PNPG1 to glucose and p-nitrophenol, which created a yellow color. The rate of increase in absorbance was measured at 405 nm [48].

Determination of intestinal amylase activities

Low intestinal amylase activity would be indicative of diminished carbohydrate and lipid digestion. Amylase hydrolyzes the α (1-4) linkage of glycogen and starch to yield D-glucose, maltose, and a resistant core of dextrin [49]. In order to determine amylase activity, the substrate p-Nitrophenyl D-maltohepatoside (PNPG7) was broken down by amylase in a series of steps to produce glucose and p-nitrophenol, which was

spectrophotometrically measured at 405 nm [48]. Intestinal mucosa amylase activities were determined using Amylase Stanbio assay kit.

Determination of serum proteins

Total protein assay kit identifies peptide bonds between enzymes using a violet color to indicate the amount of proteins. Copper ions are used to tag the carbonyl and imine groups of these proteins. The violet color produced was read at 550 nm, using the Stanbio Total Protein assay kit. Albumin protein levels were determined by using a bromocresol green (BCG) dye, to identify albumin as determined by Dumas, Waton and Biggs. Absorbance was measured at 550 nm using a Stanbio Albumin assay kit [50, 51]. The globulin levels were determined by subtracting albumin levels from total protein [16].

Determination of serum lipids

Total cholesterol and HDL levels were determined using the Stanbio Cholesterol kit and triglycerides were analyzed using the Stanbio Triglyceride kit. Total cholesterol was verified by using cholesterol esterases to hydrolyze esters to free cholesterol and fatty acids. Oxidation of the free cholesterol by cholesterol oxidase (COx) in combination with 4-aminopenzone and peroxidase formed quinoneimine chromagen that was read at 500 nm [52, 53]. HDL cholesterol levels were determined by precipitating low density lipoproteins (LDL) from serum by using the sodium phosphotungstate-Mg^{2+} method of Burnstein et al [54]. Sodium phosphotungstate solution consisted of 40 g of phosphotungstic acid per liter of a mixture of 1 M NaOH and distilled water (16/84 by volume) and 50 µL of 2 M $MgCl_2$. The solution was mixed and 2 mL of serum were added to 200 µL of sodium

phosphotungstate solution, samples were centrifuged at 1500 x g, for 30 minutes at 4 °C. Supernatant was extracted and analyzed using the Stanbio Cholesterol kit. Triglycerides were determined by using lipase action on glycerol and fatty acids. Glycerol kinase catalyzes the reaction between glycerol which was phosphorylated by adenosine-5' triphosphate (ATP) to make glycerol-3-phosphate (G-3-P) and adenosine-5'-diphosphate (ADP). Glycerylphosphate oxidase then oxidizes G-3-P to produce dihydroxyacetone phosphate (DAP) and hydrogen peroxide. Peroxidase catalyzes peroxide and 4-aminoantipyrine and 4-chlorophenol to form quinoneimine, which was spectrophotometrically read at 500 nm [55].

Determination of serum electrolytes

Total calcium and total phosphorus levels in serum were measured using Stanbio Calcium and Phosphorus assay kits. Total calcium was disassociated from proteins in an acid solution and directly reacts with ortho-cresolphthalein (OCPC). The Ca-OCPC compound formed a purple color in an alkaline solution and the concentration was measured at 550 nm [56]. Total phosphorus levels were measured by mixing phosphate ions with molybdate and the product was reduced to produce the color "molybdenum blue". Absorbance of the unreduced compound was measured at 340 nm [57].

Serum biochemical assays

Creatinine, blood urea nitrogen (BUN), uric acid, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST/GOT) Stanbio assay kits were used. Creatinine levels were determined by adding picric acid in alkaline conditions

to form a colored complex, which spectrophotometrically absorbed at 510 nm [58]. BUN levels were determined by urease, which hydrolyzed urea to yield ammonia and carbon dioxide. Salicylate, sodium nitroprusside and hypochlorite reacted with ammonium ions to create a blue green chromophore, which was measured spectrophotometrically at 600 nm [59]. Uric acid levels were determined by using uricase to form hydrogen peroxide and allantoin. Peroxidase acted upon hydrogen peroxide, 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB) and 4-aminophenazone to create a red violet quinoneimine complex that was measured at 520 nm [60, 61]. ALP levels were measured by the reaction between 4-nitrophenyl phosphate using ALP to catalyze the reaction to form 4-nitrophenol and phosphates. 4-nitrophenol was then read at 405 nm [62]. ALT levels were measured by the reaction between L-alanine and 2-oxoglutarate catalyzed by ALT to form glutamate and pyruvate. LDH then catalyzes the reaction between pyruvate and nicotinamide adenine dinucleotide (NADH) to produce lactate and the oxidation of NAD^+. The rate decrease of absorbance read at 340 nm was directly proportional to ALT activity [63, 64]. AST levels were measured by the reaction between L-aspartate and 2-oxoglututarate catalyzed by AST to yield oxaloacetate and glutamate. Formation of oxaloacetate then reacted with NADH with malate dehydrogenase (MDH) to make NAD. The rate of oxidation of NADH was measured at 340 nm [65].

2.4 Objective 2

Determination of serum leptin and insulin

Leptin rat ELISA kit [66] and Rat Insulin ELISA kit [67] were used to determine serum leptin and insulin levels. Leptin is a hormone that acts on the hypothalamus by suppressing food intake and increasing energy consumption. This assay has a specific antibody for rat leptin that is coated in the well plate provided. One hundred microliters of dilution buffer, 100 µL of standard control and 100 µL of samples were added to wells respectively and incubated for 1 hour. Leptin present in samples bound to wells by the immobilized antibody. Wells were washed and 100 µL of biotinylated anti-rat leptin antibody conjugate was added. It was incubated at room temperature for 1 hour. Biotinylated antibody was washed and 100 µL of HRP-conjugated streptavidin enzyme was added into wells and incubated for 30 minutes at room temperature. Wells were washed once more and 100 µL of TMB substrate was added to wells and incubated for 30 minutes, color development occurred if leptin was bound. One hundred microliters of Stop solution were added and the intensity of the color was measured at 450 nm within 15 minutes [66]. Insulin is a hormone that is produced by the beta cells of the pancreas. It is needed for the uptake of glucose into the cells for further metabolism. The sandwich technique used for insulin analysis consisted of two antibodies that were directed against separate antigen epitopes of the insulin molecule. Ten microliters of standards, controls and samples were added to the microwell plate. Insulin in the serum sample reacted with peroxidase-conjugated anti-insulin antibodies and the anti-insulin antibodies bind to the well. Seventy-five microliters of conjugate prepared solution were added and incubated for 2 hours at room temperature.

The washing step removed the unbound enzyme labeled antibodies. The bound conjugate was detected by reaction with 100 µL of 3,3'5,5'-tetramethylbenzidine (TMB substrate) and incubated for 15 minutes at room temperature. The reaction was stopped by adding 100 µL of stop solution and read at 450 nm within 30 minutes [67].

2.5 Objective 3

Hematological assays

After euthanatizing the rats via guillotine as approved by IACUC, (anesthetics were not used because of their potential adverse effects on some of the assay results) ~~it could compromise the blood)~~ blood was quickly collected into red and purple-capped tubes for analysis. The red-capped tubes were centrifuged and used to collect serum for further analysis of immunological activity. Blood samples collected in the purple-capped tubes were sent to a hospital lab for complete blood count analysis (CBC) using the Siemens Advia 120 systems analyzer.

White blood cell count

The white blood cell levels were determined by performing a CBC using an automated hematology analyzer. A high white blood cell count can be indicative of an infection, inflammation, or reaction to medication that could be indicative of Type 2 diabetes. Differential WBCs were analyzed for a specific interpretation of infections. Samples were sent out to a laboratory for analysis as stated previously.

Determination of antioxidant enzymes

This study investigated the presence of enzymes using various assays that included Catalase Assay kit, Rat Superoxide Dismutase (SOD) assay and Reduced Glutathione (GSH) assay. The specimen that was used for these assays was serum. Catalase is an enzyme that detoxifies reactive oxygen species such as hydrogen peroxide from liver, kidneys and erythrocytes [68]. SOD and GSH are enzymes that are used to prevent diseases that are caused by oxidative stress [69]. Enzymes that are indicative of lipid degradation due to oxidative stress in diabetic patients are Superoxide Dismutase (SOD) and Catalase (CAT). SOD activity was assayed using the method of Marklund and Marklund [70] it determined the ability of SOD to stop the autoxidation of pyrogallol by 50%. The assay mixture of one milliliter contained 500 µl of 0.1M sodium phosphate buffer, 32 µL of 3.3 mM EDTA, 60 µL of 8.1 mM pyrogallol and an appropriate amount of serum that contained 7–10 µg of protein. The change in absorbance at 420 nm of the assay mixture was observed for two minutes at 25°C against a blank that contained all the ingredients with the exception of the tissue homogenate. The CAT assay was based on the method by Aeibi [23]. It was a mixture of one milliliter containing 500 µl of 0.1 M sodium phosphate buffer, 100 µl of hydrogen peroxide, and 20 µl of homogenate treated with Triton X-100 (diluted to 1% with double DI water). An equal part of the serum was mixed with diluted Triton X-100 and 20 µl of the resulting homogenate was used for this assay. The decrease in absorbance was then read at 240 nm for five minutes at 25°C against a blank containing all the ingredients without the serum sample. The reduced glutathione (GSH) assay was conducted using Ellman's method [71]. The serum was mixed with 10% trichloroacetic acid (TCA) and was centrifuged at 3,000 x g. The supernatant (1 mL) was treated with 0.5

mL of Ellman's reagent [19.8 mg of 5,5'-dithiobisnitro benzoic acid (DNTB) in 100 mL of 0.1% 0.2M phosphate buffer (pH 8.0)]. The absorbance was then read at 412 nm.

Determination of lipid peroxidation

Free radicals are unstable molecules that can mutate DNA, proteins and lipids. Oxidative damage in the cell membrane is an example of lipid peroxidation. Increased levels of peroxidation in diabetic patients are indicative of the development of cellular dysfunction, hypertension, cardiovascular disease, and hypercoagulability [72]. The development of lipid peroxides was measured in the serum. The formation of MDA, an end product of fatty acid peroxidation was measured spectrophotometerically at 532 nm by using a thiobarbituric acid reactive substance (TBARS) as described by Genet et al. [73]. The final reaction mixture of three milliliters contained, 1.5 mL of 10 mM potassium phosphate buffer (pH 7.4), 0.5 mL of the serum, 0.5 mL of 30% trichloroacetic acid (TCA) and 0.5 mL of thiobarbituric acid (TBA) (0.53%). The mixture was then heated for one hour at 80°C and cooled. The contents were centrifuged at 2,700 x g for five minutes at 4°C and the absorbance was measured in the cleared supernatant at 532 nm against a blank.

Determination of enzymes of lipid and carbohydrate metabolism in liver and kidney

A decrease in enzymatic activities, specifically, glycolytic and pentose phosphate pathways, as well as an increase in the activities of gluconeogenic, glycogenolytic and lipolytic pathways is indicative of Type 2 diabetes [74]. Measuring the activities of the following enzymes provided the information necessary to understand enzymatic activities.

Pyruvate kinase, glucose-6-phosphate dehydrogenase (G6PD), and malic enzymes were assayed using the methods discussed below.

Preparation of liver and kidney homogenates

The extraction buffer for liver was made up of 60 mM sucrose, 220 mM manitol, 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 5 mM dithiothreitol (1:10 w/v). Liver and kidney samples were weighed and homogenized with the above buffer. Homogenates were centrifuged for 20 minutes and the supernatant used in the assays for the concentration of enzymes.

Pyruvate kinase

Pyruvate made by pyruvate kinase reacts with lactate dehydrogenase and the oxidation of NADH was used to determine activity of this enzyme [75]. The assay mixture contained 0.98 mL of 100 mM Tris-HCl buffer, pH 7.5, 5 mM phenolpyruvate, 5 mM ADP, 0.2 mM NADH, 10 mM $MgCl_2$, 100 mM KCl and excess lactate dehydrogenase. The reaction was started by the addition of enzyme homogenate (0.02 mL). The activity was then normalized to the protein concentration in the sample. Protein concentrations were determined using the Stanbio Total Protein assay kit, as previously discussed.

Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase is part of the pentose phosphate pathway. This assay mixture contained 0.98 mL of 100 mM Tris-HCl buffer, pH 8.0, 1.0 mM glucose-6-phosphate, 0.5 mM $NADP^+$ and 5.0 mM $MgCl_2$. The reaction was started by the addition

of 0.02 mL enzyme homogenate. It was mixed immediately and the increase in absorbance was measured at 340 nm recorded for approximately five minutes [75].

Malic enzyme

Malic enzyme activity was assayed by the adjustment of the methods of Storey and Bailey [75]. The assay mixture contained 0.98 mL of 100 mM Tris-HCl buffer pH 7.5, 1.0 mM malate, 0.3 mM $NADP^+$ and 0.5 mM $MnCl_2$. Adding 0.02 mL enzyme homogenate to the assay mixture in a 3-mL quartz cuvette started the reaction. Readings were taken at 340 nm for one minute to detect any endogenous reducing activity. To start the second reaction, 0.1 mL of 0.3 M L-malate was added to the cuvette. Readings were taken again after another minute at 340 nm. The change in optical density as $NADP^+$ was reduced to NADPH after adding the malate, minus the change due to endogenous activity; this was then used for calculation of malic enzyme activity (nanomoles of $NADP^+$ reduced per minute). Glycolysis uses malic enzyme (ME) to convert malate into pyruvate, yielding NADPH. The specific protein in the sample was then correct for the activity.

Serum Cytokine determination

This study investigated the presence of cytokines using interleukin-6 (IL-6) ELISA kit [76]. The specimen that was used for these assays was serum. IL-6 production is induced by antigens such as cytokines, bacterial and viral infections. It is involved in the pathogenesis of many diseases. An anti-rat IL-6 coating antibody was adsorbed onto the wells of the plates. One hundred microliters of assay buffer were added to blank wells and 50 µL of assay buffer to sample wells along with 50 µL of sample. Rat IL-6 that was present in the

sample or standard bound to antibodies adsorbed to the wells. Fifty microliters of biotin-conjugated anti-rat IL-6 antibody was added and bound to rat IL-6 captured by the first antibody. After a 2-hour incubation the unbound biotin-conjugated anti-rat IL-6 antibody was removed during the wash step. One hundred microliters of streptavidin-HRP was then added and bound to the biotin-conjugated anti-rat IL-6 antibody. After the next 1-hour incubation unbound Streptavidin-HRP was removed during the wash step and 100 µL of TMB substrate was then added to the wells. A colored product was formed in proportion to the amount of rat IL-6 present in the sample or standard. The reaction was terminated by the addition of 100 µL of stop solution (acid) and absorbance was measured at 450 nm [76].

2.6 Statistical Analysis

Results are presented as means ± SEM. Analysis of variance (ANOVA) was used to test for differences among the groups. A post-hoc analysis was carried out using the Duncan's multiple range test for significant difference among the means (P <0.05).

3. Results and Discussion

Objective 1

3.1 Body and Organ Weight Changes, Food and Fluid Intake

Throughout the 28 days of treatment, body weight, food intake, and fluid consumption was evaluated. The *K. pinnata* treated group consumed the same amount of food as the diabetic control group but weighed significantly ($P < 0.05$) less (Figure 4 and 5). Studies conducted in our laboratory previously established that with the consumption of aqueous preparation of *K. pinnata* rats lost weight, although results were insignificant [40]. We would like to note that our previous study duration was 30 days and *K. pinnata* extract was provided via water bottles in comparison to our current study which was 28 days of treatment excluding the 21 days to develop Type 2 diabetes and administration of treatment was via gavage, this could explain the deviation in our results. Hil et al. reported that quercetin, a phytochemical found in *K. pinnata,* decreases high-fat diet induced body weight gain [38, 39]. This could explain why the diabetic rats administered *K. pinnata* extract lost a significant ($P < 0.05$) amount of weight compared to the diabetic control group. Fluid consumption (Figure 6) indicated that there was no significant ($P > 0.05$) difference between the diabetic *K. pinnata* treated group in comparison to the diabetic control group. There was a significant ($P<0.05$) decrease in fluid consumption for the diabetic Metformin treated group compared to the diabetic control. The diabetic *K. pinnata* and diabetic Metformin treated groups had a significant ($P < 0.05$) increase in kidney weight in comparison to the control group. It was also found that the diabetic

control, diabetic *K. pinnata*, and diabetic Metformin treated groups had a significant ($P < 0.05$) increase in liver and intestinal weight in comparison to the control group (Figure 7). Diabetic nephropathy is thought to be caused by the metabolic, and glomerular disruption [77]. Non-alcoholic fatty liver disease (NAFLD) is common in Type 2 diabetic patients due to insulin resistance and obesity [78]. Type 2 diabetes also impacts the digestive system in the form of postprandial hyperglycemia, it slows the emptying of digested contents, due to high blood glucose levels after a meal [79]. These factors could potentially play a role in the increase of kidney, liver and intestinal weight.

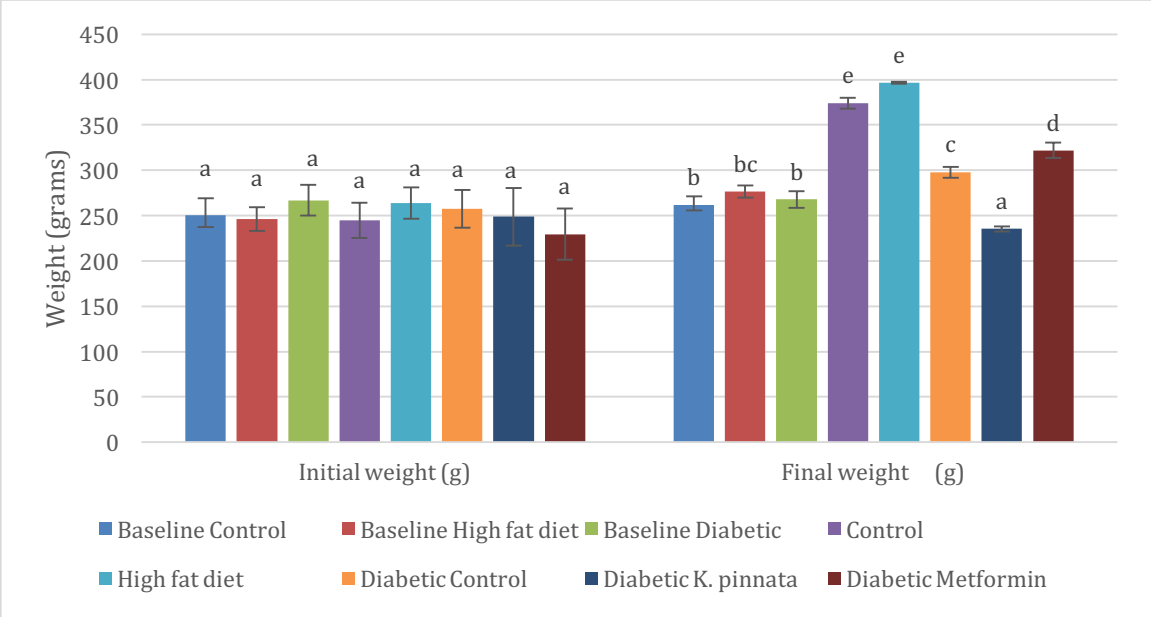

Figure 4. Body Weight Changes of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

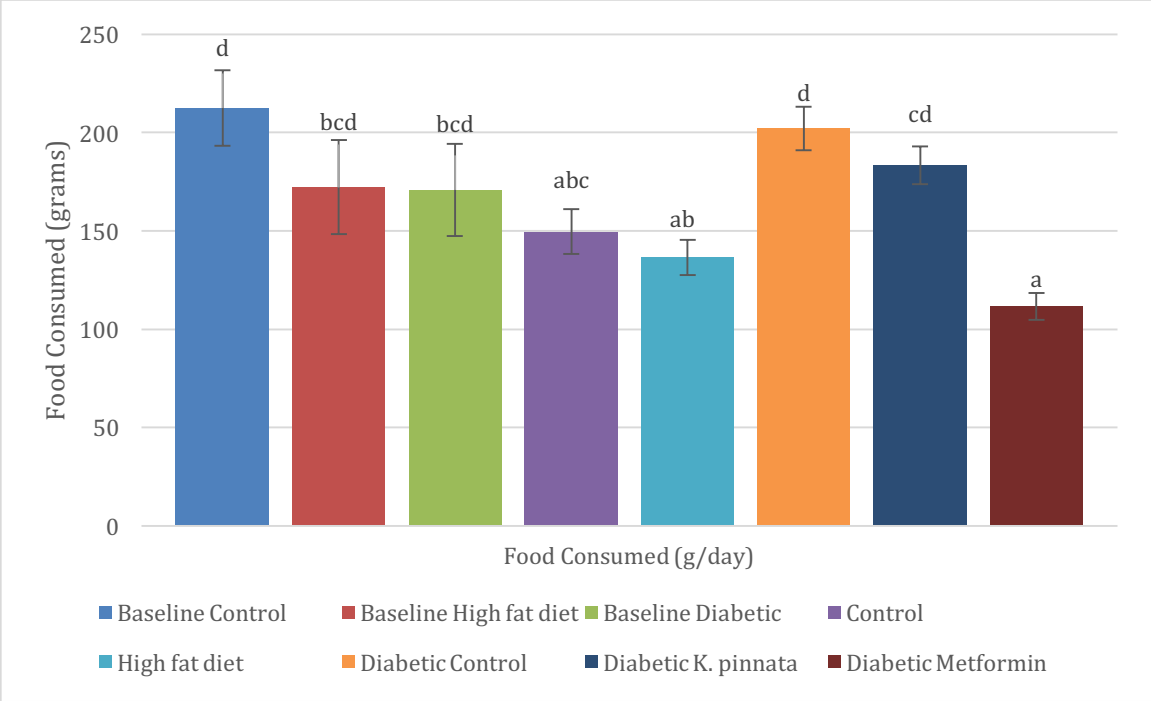

Figure 5. Food Intake of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

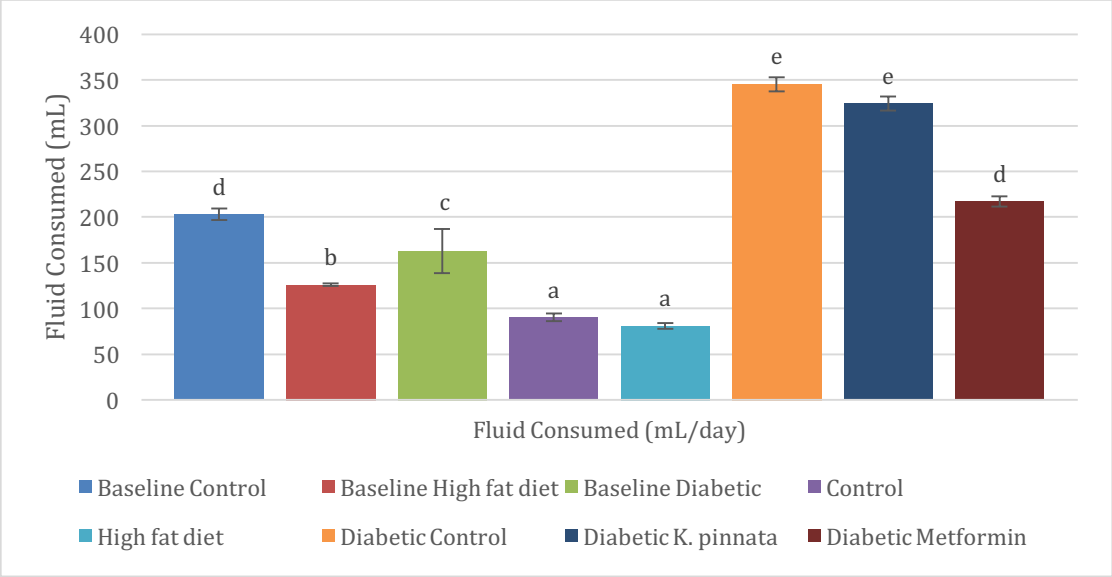

Figure 6. Fluid Consumption of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

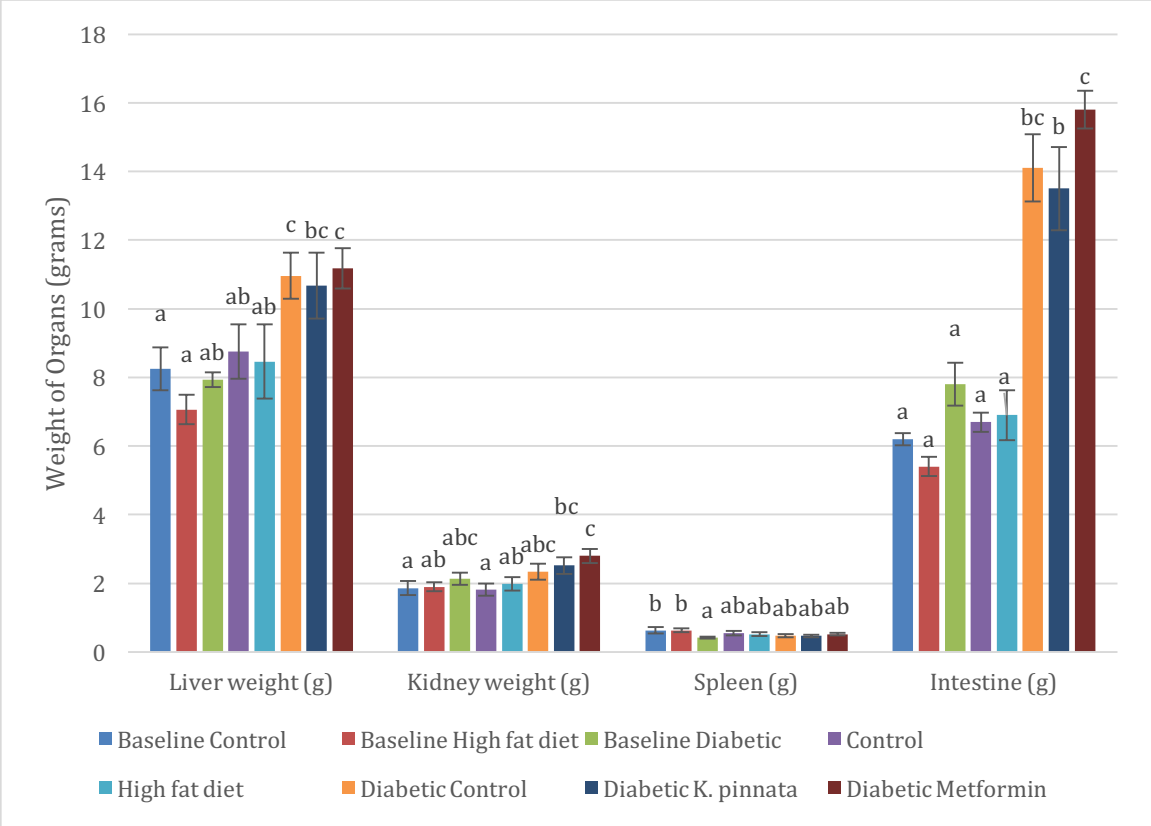

Figure 7. Average Organ Weight Changes of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

3.2 Blood Glucose

Accumulation of glucose in the blood stream is associated with diabetes [13]. There was a significant ($P < 0.05$) decrease in the diabetic *K. pinnata* and diabetic Metformin treated groups in comparison to the diabetic control group (Figure 8). The Metformin treated group did not significantly reduce blood glucose levels in comparison to the diabetic *K. pinnata* treated group. There was no significant ($P > 0.05$) difference between the baseline diabetic group, diabetic *K. pinnata* group and the diabetic Metformin treated group. Previous studies from our lab support this finding, blood glucose levels significantly decreased for Type 1 diabetic rats treated with *K. pinnata* extract [40]. Another study showed that dichloromethane (DCM) leaf fraction of *K. pinnata* was effective in the anti-hyperglycemic treatment of streptozotocin-induced diabetic rats. The study found that the promotion of insulin secretion was glucose independent and K^+-ATP channel dependent. The phytochemical component of the *K. pinnata* DCM fraction was found to be a derivative of phenyl alkyl ether [80]. These results are indicative that aqueous preparation of *K. pinnata* contains a phytochemical that may aid in the reduction of blood glucose through the enhancement of insulin secretion.

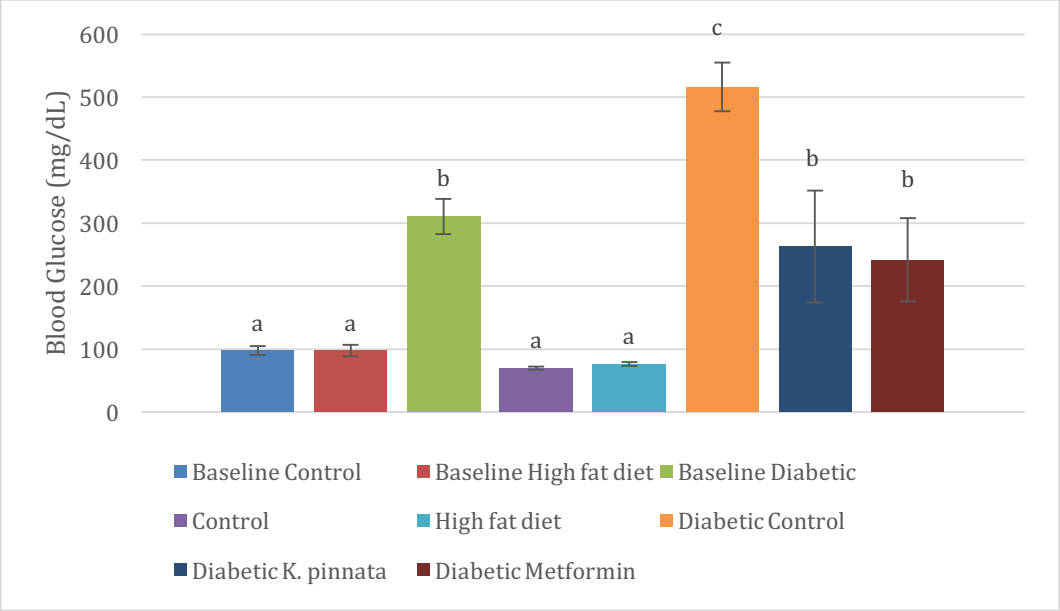

Figure 8. Blood Glucose levels in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

3.3 Serum and Intestinal Amylase

There was no significant ($P > 0.05$) difference among the groups for amylase activity in the serum of Type 2 diabetic rats. However, there was a non-significant ($P > 0.05$) increase in serum amylase for the diabetic *K. pinnata* treated group compared to the diabetic control and Metformin treated groups (Figure 9). This may indicate that aqueous preparation of *K. pinnata* may contain components that increase the production of amylase through the restoration of insulin that work closely with the acinar cells of the pancreas. Amylase activity in the proximal intestinal mucosa showed a significant ($P < 0.05$) increase in the high fat diet group in comparison to all the groups (Figure 10). The high fat diet group increased amylase activity in the proximal intestinal mucosa correlates with the normal function of the pancreas to release digestive enzymes during the consumption of foods high in fat [4]. For the amylase activity in the distal intestinal mucosa it was noted that there was no significant ($P < 0.05$) difference between the diabetic *K. pinnata* treated group and the diabetic control group, although there was a significant ($P < 0.05$) increase in the baseline diabetic group compared to the diabetic *K. pinnata* treated group (Figure 11). This could be due to the normal production of amylase in the baseline diabetic group because it is in the early stages of Type 2 diabetes [4].

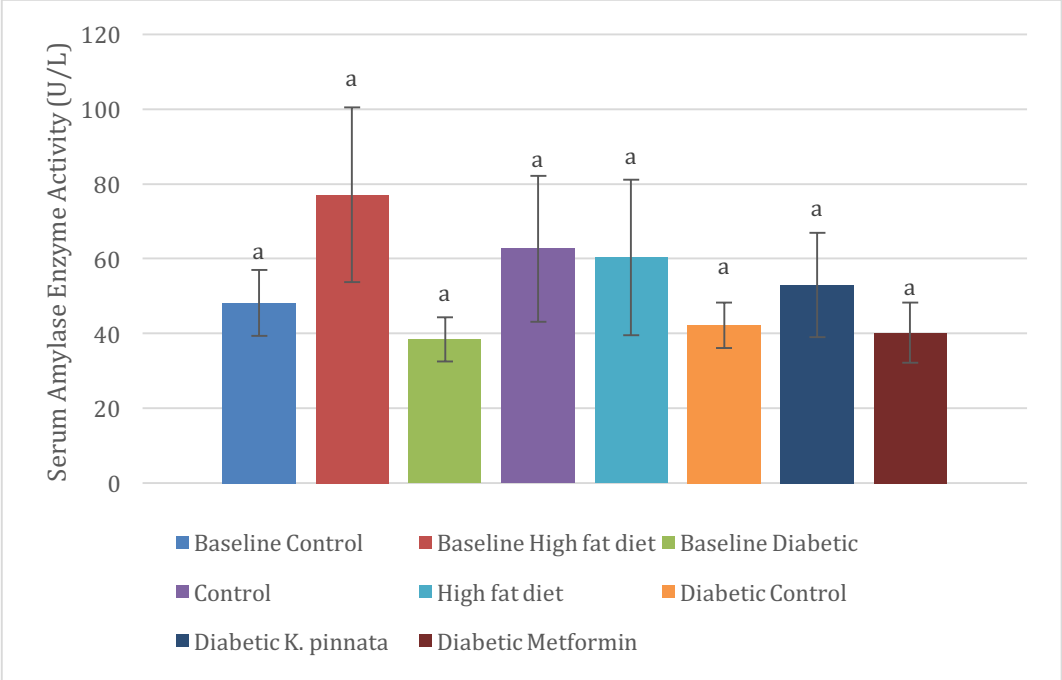

Figure 9. Amylase Activity in the Serum of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Values were not significantly different among the groups ($P < 0.05$)

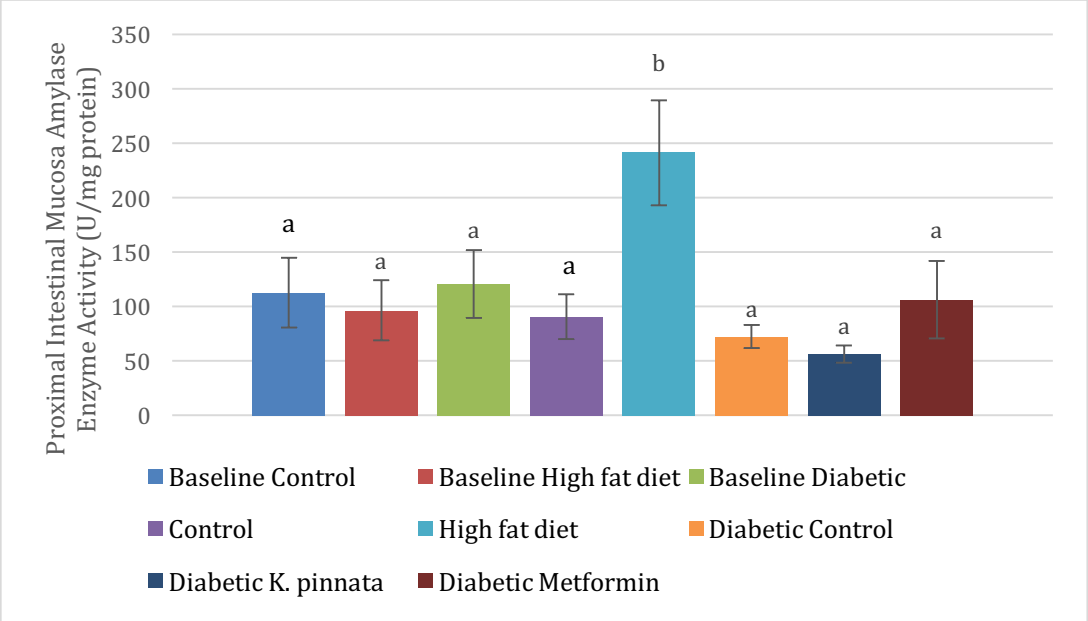

Figure 10. Amylase Activity in the Proximal Intestinal Mucosa of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

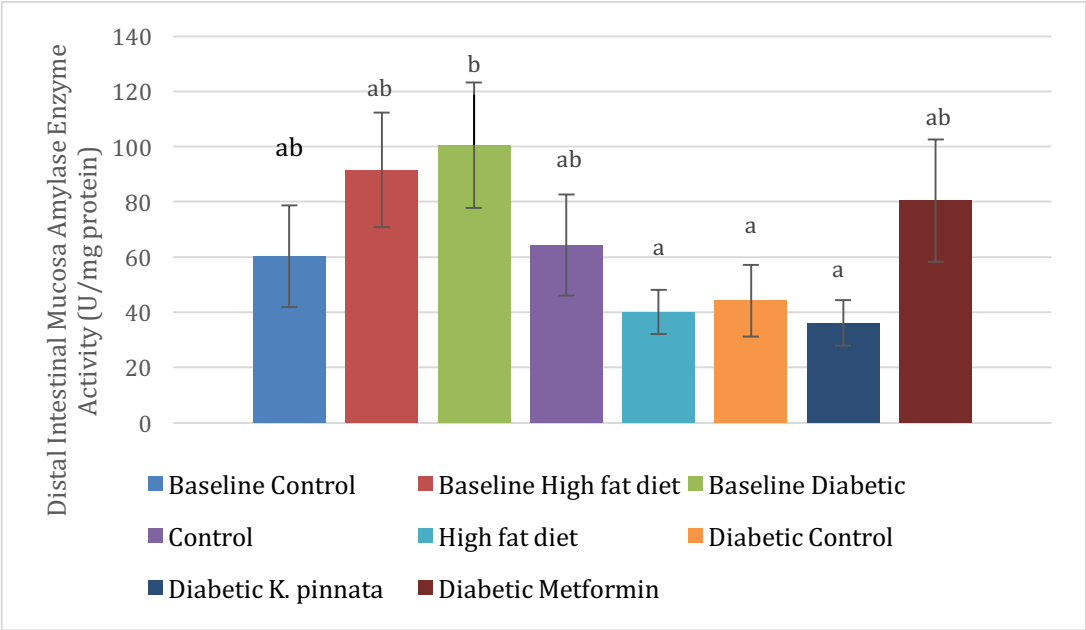

Figure 11. Amylase Activity in the Distal Intestinal Mucosa of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

3.4 Serum Proteins

In this study, serum albumin, globulin and total protein levels were significantly ($P < 0.05$) decreased in the baseline diabetic group, in comparison to the control group. There was a significant ($P < 0.05$) increase in globulin and total protein for the diabetic *K. pinnata* treated group in comparison to the baseline diabetic. There was no significant ($P > 0.05$) difference between the diabetic *K. pinnata* treated and the control group. There was a non-significant ($P > 0.05$) increase in albumin for the diabetic *K. pinnata*, and diabetic Metformin treated group in comparison to the baseline diabetic group (Figure 12a). These findings may be indicative of the decrease in protein glycation of albumin, globulin and total protein due to the hypoglycemic effects of diabetic *K. pinnata* and diabetic Metformin. There was a significant ($P < 0.05$) increase in the albumin:globulin ratio for the baseline diabetic group in comparison to the diabetic *K. pinnata* and diabetic Metformin treated groups (Figure 12b). Our data indicates that albumin:globulin ratio was lowered in the diabetic rats with treatment of diabetic *K. pinnata* and diabetic Metformin although the finding was not significant.

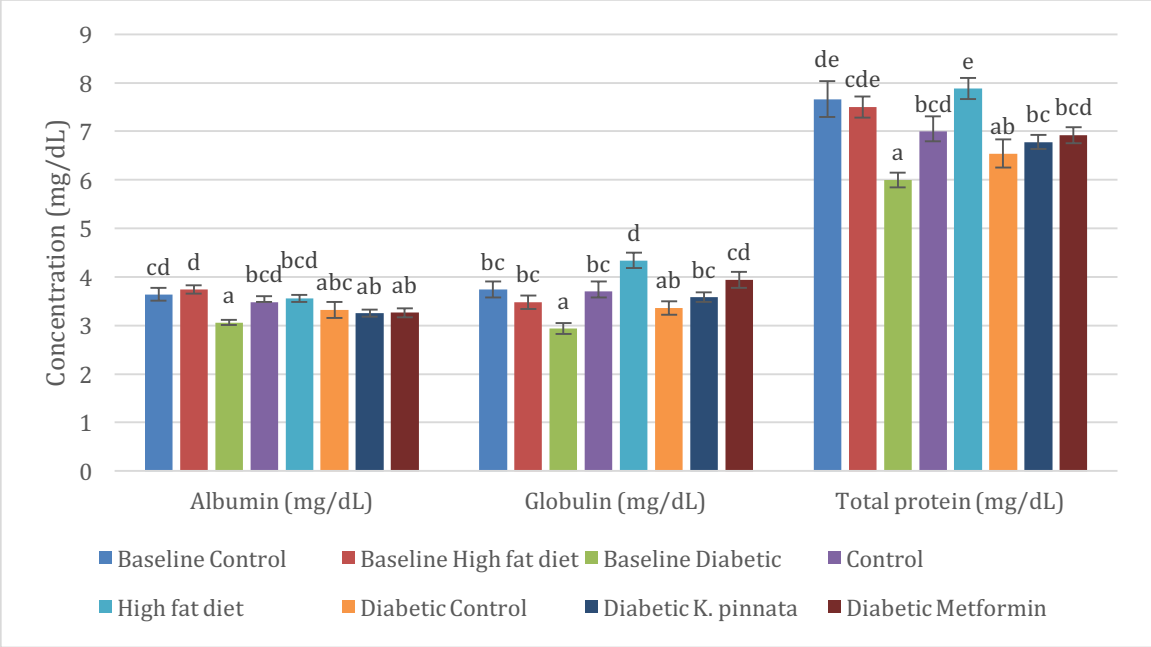

Figure 12a. Serum Protein Profile in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

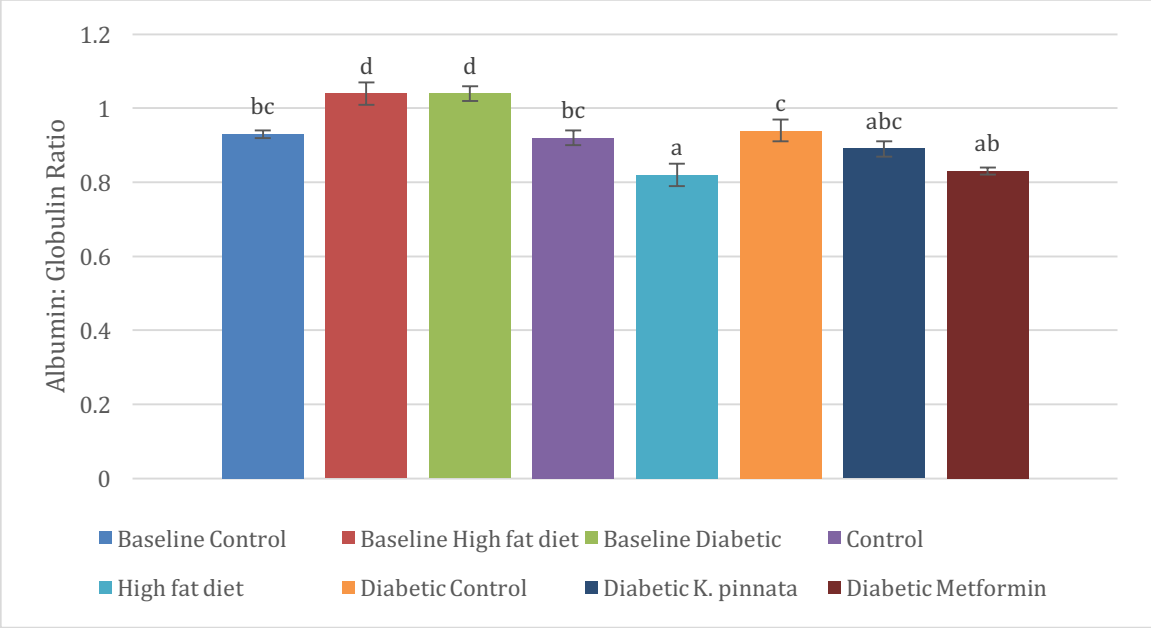

Figure 12b. Albumin:globulin ratio in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter scripts are significantly different ($P < 0.05$).

3.5 Serum Lipid Profile

In this study, there was no significant ($P > 0.05$) difference in serum cholesterol levels between the control and the diabetic *K. pinnata* treated group. There was a significant ($P < 0.05$) increase in HDL levels in diabetic *K. pinnata* treated group compared to the diabetic control group. Serum triglyceride levels were significantly ($P < 0.05$) reduced in the diabetic *K. pinnata* treated group in comparison to the diabetic control group. It was noted that there was no significant ($P > 0.05$) difference between the triglyceride levels of the diabetic Metformin treated group and the diabetic control group (Figure 13). This may be indicative that aqueous preparation of *K. pinnata* effective metabolism of lipids due to insulin restoration (Figure 14).

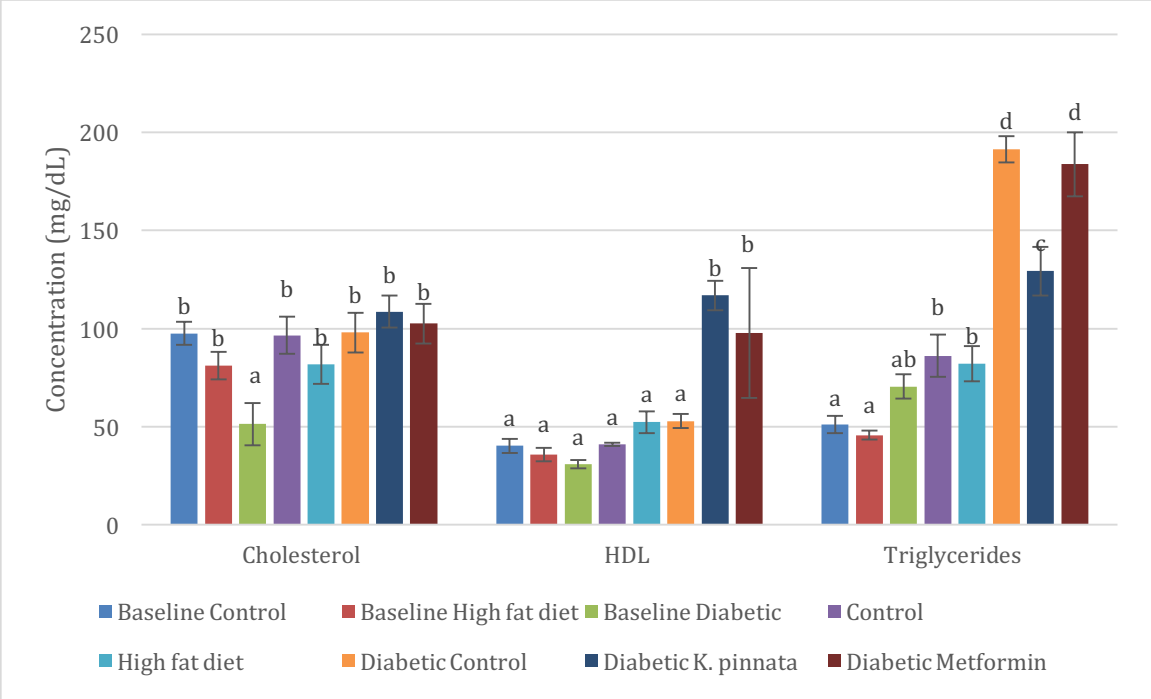

Figure 13. Effect of Aqueous *Kalanchoe pinnata* Preparation on Serum lipid profile. Figures that share different letter superscripts are significantly different ($P < 0.05$).

3.6 Serum Electrolytes

Serum electrolytes analyzed in this study were phosphorus and calcium. There was a significant ($P < 0.05$) increase in phosphorus levels of the diabetic *K. pinnata* treated group in comparison to the diabetic control group. Our findings suggest that the consumption of aqueous preparation of *K. pinnata* may repair phosphorus metabolism due to hypoglycemic activity, which may increase intracellular phosphate. Calcium levels were not significantly alerted among groups (Figure 14).

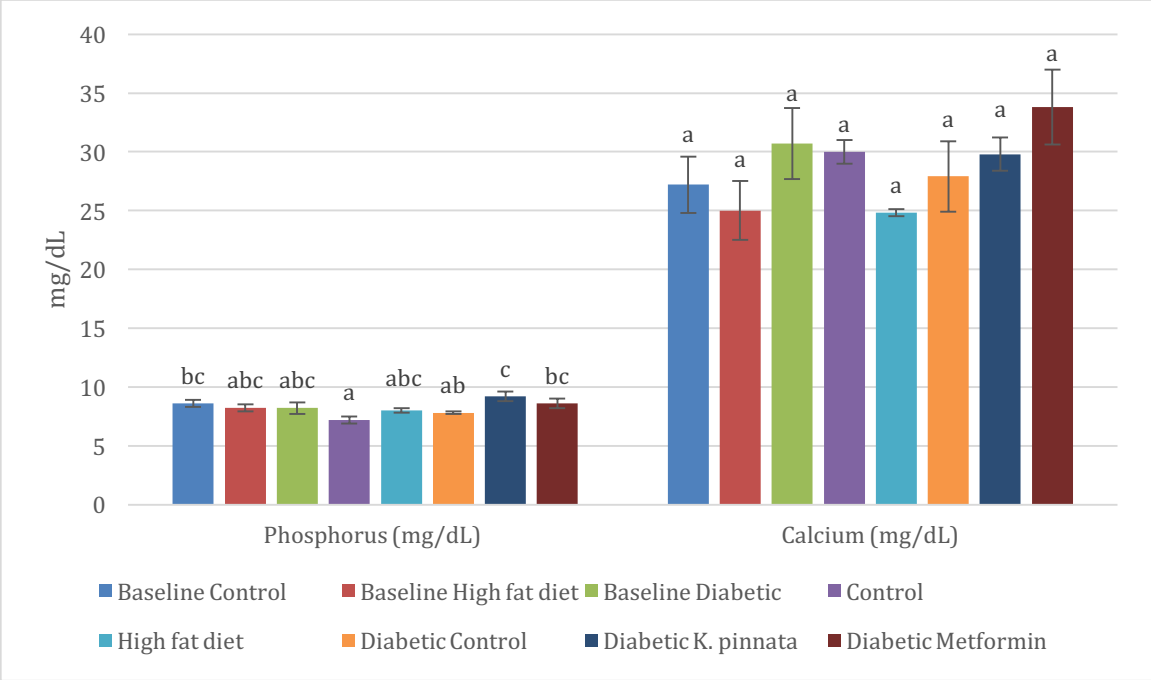

Figure 14. Serum Electrolytes in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata*. Figures that share different letter superscripts are significantly different ($P < 0.05$).

3.7 Serum Biochemical assays

High serum levels of liver enzymes such as alkaline phosphatase (ALP) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are indicative of liver damage. Kunustor et al. [81] reported that increased levels of AST were associated with the amplified risk of Type 2 diabetes. Liver enzymes analyzed in the serum of Type 2 diabetic rats were ALP, ALT and AST. There was a significant ($P < 0.05$) increase of ALP and ALT activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. This could be indicative of adverse effects of the aqueous preparation of *K. pinnata* consumption on the liver. There was a non-significant ($P > 0.05$) decrease in AST activity in the diabetic *K. pinnata* group compared to the diabetic control group (Figure 15). Blood urea nitrogen, creatinine and uric acid, increased levels of these contents are indicative of kidney disease [82, 83]. It was noted that there was a significant ($P < 0.05$) increase in blood urea nitrogen of the diabetic control, diabetic *K. pinnata*, and diabetic Metformin treated groups compared to the control. There was a non-significant ($P > 0.05$) increase in creatinine levels in the diabetic *K. pinnata* treated group compared to the diabetic control group. It was also noted that there was a significant ($P < 0.05$) increase in uric acid levels in the diabetic *K. pinnata* group compared to the diabetic control group (Table 2). These findings indicate that *K. pinnata* may adversely affect proper kidney function in Type 2 diabetic rats.

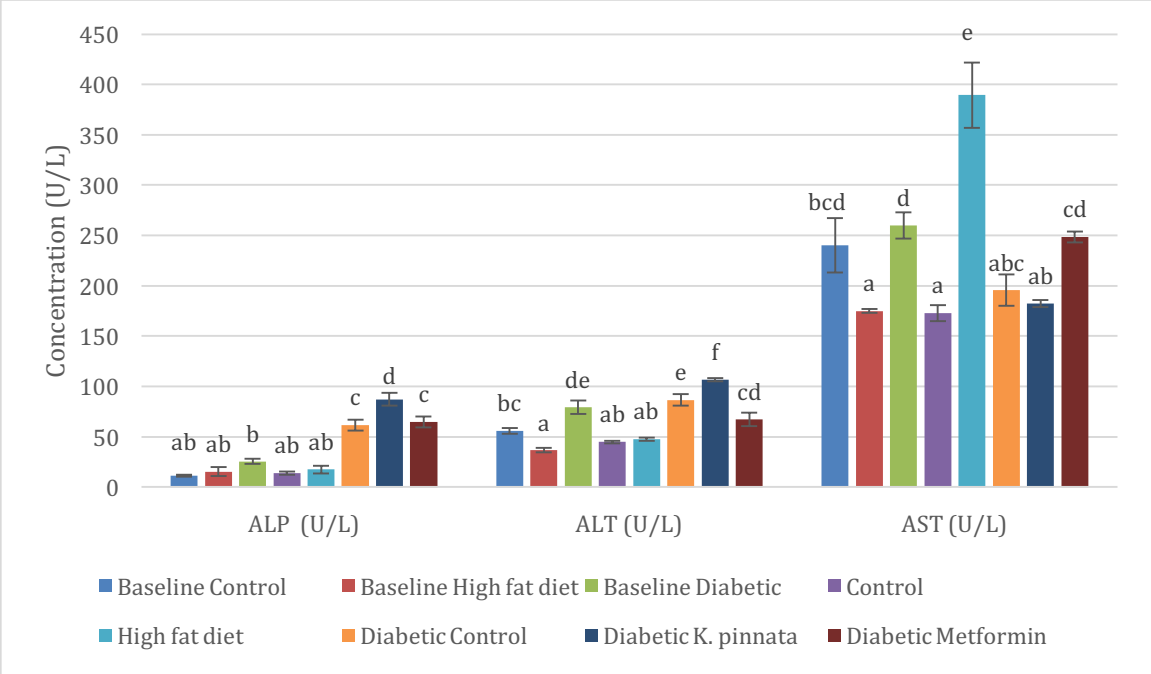

Figure 15. Liver Function Enzymes in the Serum of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter superscripts are significantly different ($P < 0.05$). ALP= Alkaline Phosphatase, ALT = Alanine Amino Transferase, AST = Aspartate Amine Transferase.

Table 2. Kidney Function Indices in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata*.

	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Baseline Control	22.20 ± 1.77^{a}	0.32 ± 0.04^{ab}	2.62 ± 0.14^{bc}
Baseline High fat diet	17.20 ± 0.97^{a}	0.42 ± 0.06^{b}	2.64 ± 0.08^{bc}
Baseline Diabetic	17.60 ± 2.20^{a}	0.34 ± 0.02^{ab}	1.55 ± 0.38^{ab}
Control	22.80 ± 3.54^{a}	0.30 ± 0.03^{ab}	1.15 ± 0.03^{a}
High fat diet	23.00 ± 1.14^{a}	0.34 ± 0.02^{ab}	1.78 ± 0.15^{ab}
Diabetic Control	47.60 ± 2.37^{b}	0.24 ± 0.04^{a}	1.31 ± 0.50^{ab}
Diabetic *K. pinnata*	54.20 ± 5.26^{b}	0.28 ± 0.02^{a}	3.86 ± 0.16^{c}
Diabetic Metformin	53.75 ± 5.27^{b}	0.28 ± 0.03^{a}	1.25 ± 0.13^{ab}

Values that share different letter superscripts are significantly different ($P < 0.05$).

Objective 2

3.8 Serum Leptin and Insulin

Serum leptin and insulin levels were investigated in this study. There was a significant ($P < 0.05$) increase in leptin levels in the baseline high fat diet group compared to the baseline diabetic group. There was a non-significant ($P > 0.05$) increase of leptin in the diabetic *K. pinnata* treated group compared to the diabetic control group (Figure 16). This may suggest that the aqueous preparation of *K. pinnata* may increase the secretion of leptin which may explain the observed weight reduction in the diabetic *K. pinnata* treated rats. Insulin levels indicated that there was a significant ($P < 0.05$) increase in the diabetic Metformin treated group compared to the diabetic control group. There was a non-significant ($P > 0.05$) increase in the diabetic *K. pinnata* treated group compared to the baseline diabetic and diabetic control group (Figure 17). This may suggest that the aqueous preparation of *K. pinnata* may increase the levels of insulin by repairing the beta-cells of the islets within the pancreas. Further studies are needed to evaluate the effects of long-term consumption of *K. pinnata* aqueous extract preparation on insulin levels in Type 2 diabetes.

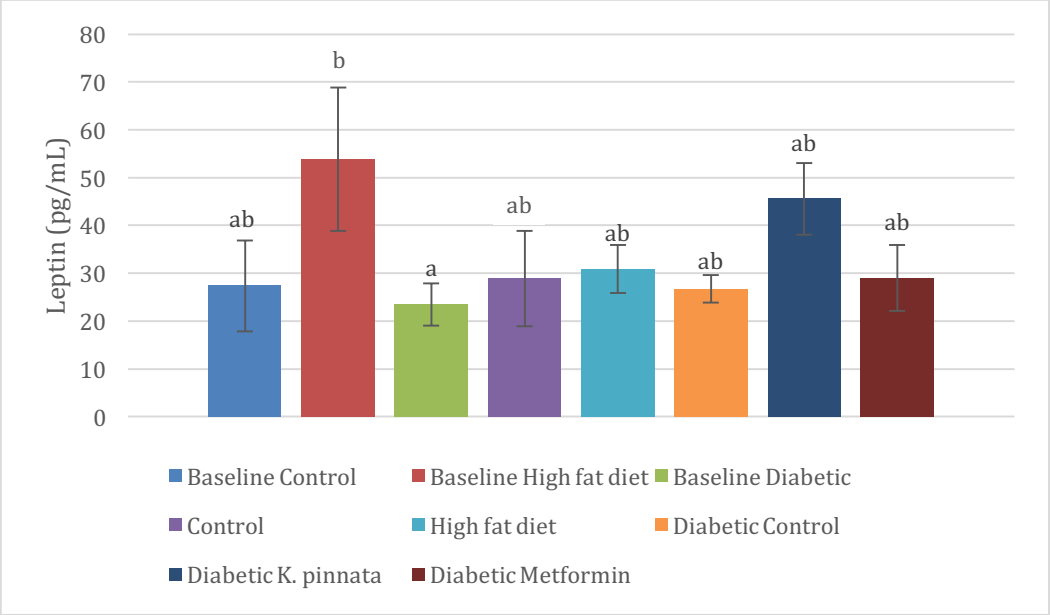

Figure 16. Effect of Aqueous *Kalanchoe pinnata* Preparation on Serum Leptin in Type 2 Diabetic Rats. Figures that share different letter superscripts are significantly different ($P < 0.05$).

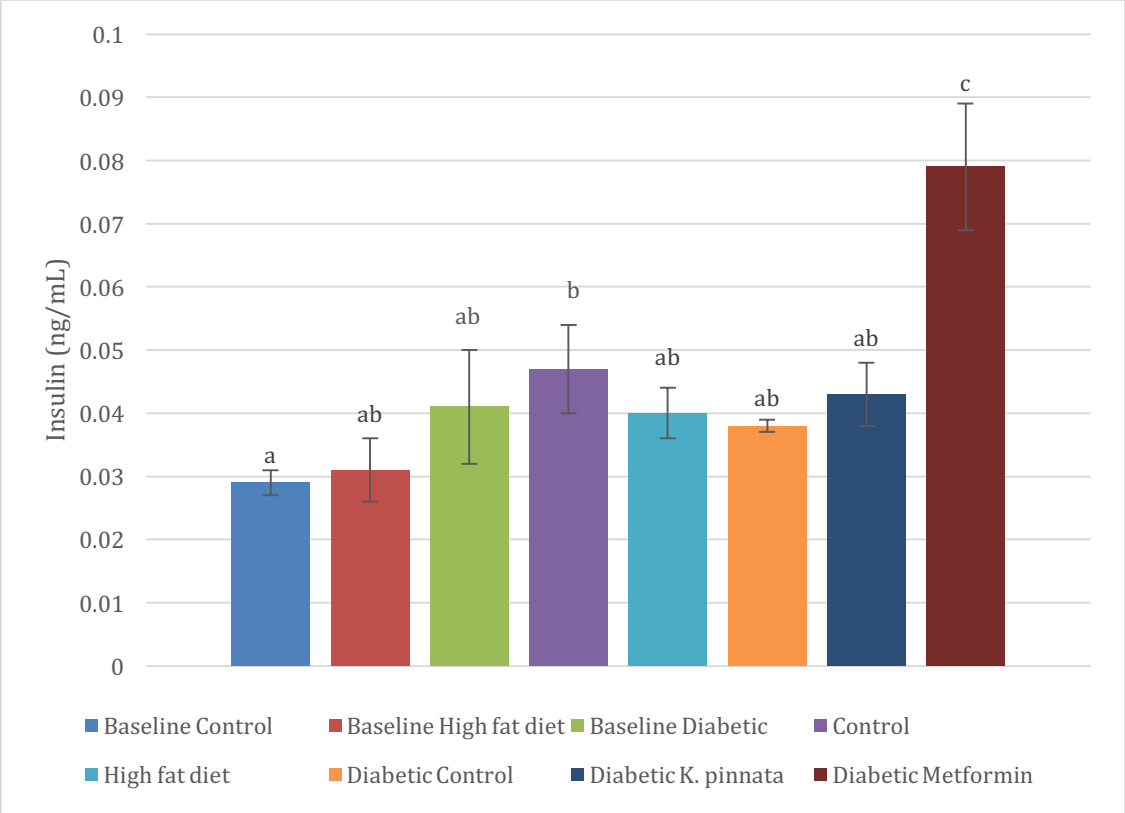

Figure 17. Effect of Aqueous *Kalanchoe pinnata* Preparation on Serum Insulin in Type 2 Diabetic Rats. Figures that share different letter superscripts are significantly different ($P < 0.05$).

Objective 3

3.9 Complete Blood Count

In this study, there was a significant ($P < 0.05$) increase in WBCs in the diabetic *K. pinnata* treated group compared to the diabetic control group. It was noted that there was no significant ($P > 0.05$) difference between the WBC count of the control and the diabetic *K. pinnata* treated group. There was no significant ($P > 0.05$) difference in RBC levels between the control and the diabetic *K. pinnata* treated group. Similarly, there was no significant ($P > 0.05$) difference between the RBC levels in the diabetic control group and the diabetic *K. pinnata* treated group. There was a significant ($P < 0.05$) increase in the HGB levels in the diabetic *K. pinnata* treated group compared to the baseline control, control and diabetic control group. It was also noted that there was no significant ($P > 0.05$) difference between the control and the diabetic control group (Table 3). The increase in RBC and HGB levels of the *K. pinnata* treated groups could be indicative of an attempt to recover normal microvascular functionality, and adequate blood oxygen supplies to the pancreas, it may be attempting to compensate for the disease. Xu et al. [84] reported that there was a significant decrease in basophils and eosinophils, and a nonsignificant decrease in lymphocytes for patients with diabetic ketoacidosis. Monocytes and neutrophils were reported to significantly increase due to deterioration in glycemic control. In this study, basophil levels were not significantly ($P > 0.05$) altered among the groups. It was noted that there was a significant ($P < 0.05$) decrease of eosinophils in the diabetic *K. pinnata* treated group compared to the diabetic control

group. There was no significant ($P > 0.05$) difference in eosinophil levels in the diabetic *K. pinnata* treated and the control group. Similarly, there was no significant ($P > 0.05$) difference in lymphocyte levels in the diabetic control and the diabetic *K. pinnata* treated groups. However, there was a significant ($P < 0.05$) increase of lymphocytes in the diabetic control and diabetic *K. pinnata* treated group compared to the baseline control group. There was no significant ($P > 0.05$) difference in monocyte levels in the diabetic *K. pinnata* treated and the diabetic control group. There was however a significant ($P < 0.05$) increase in monocytes of the diabetic *K. pinnata* treated group compared to the baseline control group. There was no significant ($P > 0.05$) difference in monocytes levels in the control group and the diabetic *K. pinnata* treated group. There was a significant ($P < 0.05$) decrease in neutrophil levels in the diabetic *K. pinnata* treated group compared to the diabetic control and diabetic Metformin group. We also noted no significant ($P > 0.05$) difference in neutrophil levels in the control and the diabetic *K. pinnata* treated group (Table 4). The non-significant ($P > 0.05$) increase in basophils, lymphocytes and significant ($P < 0.05$) decrease in neutrophils in diabetic rats treated with aqueous preparation of *K. pinnata* may be indicative of the restoration of glycemic control. These findings need to be further investigated in order to determine the extent of the benefits and adverse effects of aqueous preparation of *K. pinnata.*

Table 3. Blood Cell Counts in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.*

	WBC (cells/uL)	RBC (cells/uL)	HGB (g/dL)
Baseline Control	3.6E+03± 1.6E+02[a]	8.3E+06 ± 3.1E+05[a]	15.8 ± 0.3[a]
Baseline High fat diet	4.8E+03 ± 4.4E+02[ab]	8.1E+06 ± 4.1E+05[a]	15.8± 0.5[a]
Baseline Diabetic	4.3E+03 ± 6.7E+02[a]	8.5E+06 ± 3.2E+05[ab]	16.4 ± 0.5[a]
Control	7.1E+03± 2.0E+02[cd]	8.6E+06 ± 3.5E+05[ab]	15.9 ± 0.4[a]
High fat diet	6.7E+03 ± 2.5E+02[cd]	8.7E+06 ± 1.9E+05[ab]	16.2 ± 0.2[a]
Diabetic Control	4.4E+03 ± 2.5E+02[a]	8.4E+06 ± 2.5E+05[ab]	16.1 ± 0.2[a]
Diabetic *K. pinnata*	6.2E+03 ± 4.5E+02[bc]	9.3E+06 ± 1.9E+05[b]	17.8 ± 0.3[b]
Diabetic Metformin	8.0E+03 ± 5.6E+02[d]	8.8E+06 ± 3.4E+05[ab]	16.6 ± 0.4[ab]

Values that share different letter superscripts are significantly different ($P < 0.05$).

Table 4. Complete White Blood Cell differential in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata*.

	Basophils (cells/uL)	Eosinophils (cells/uL)	Lymphocytes (cells/uL)	Monocytes (cells/uL)	Neutrophils (cells/uL)
Baseline Control	20 ± 4.4[a]	90.0 ± 8.3[a]	1796.0 ± 86.9[a]	556.0 ± 47.3[a]	1030.0 ± 67.3[bc]
Baseline High fat diet	25 ± 5[a]	107.5 ± 8.5[ab]	3130 ± 421.9[b]	543.3 ± 74.2[a]	1315.0 ± 68.6[c]
Baseline Diabetic	38 ± 8[a]	110.0 ± 20.2[ab]	2323.3 ± 276.0[ab]	1255.0 ± 328.5[bc]	1100.0 ± 80.8[bc]
Control	27.5 ± 4.7[a]	226.6 ± 24.0[c]	4655.0 ± 255.0[c]	1070.0 ± 70.0[ab]	556.6 ± 104.9[a]
High fat diet	27.5 ± 8.5[a]	132.5 ± 26.2[ab]	4410 ± 220.5[c]	813.3 ± 124.4[ab]	1026.6 ± 128.3[bc]
Diabetic Control	30 ± 10.8[a]	375.0 ± 75.0[d]	3070.0 ± 482.6[b]	1132.5 ± 145.3[ab]	1265 ± 185[bc]
Diabetic *K. pinnata*	43.3 ± 8.8[a]	183.3 ± 63.5[bc]	3092.5 ± 275.6[b]	1362.5 ± 75.5[bc]	400.0 ± 60.0[a]
Diabetic Metformin	26.6 ± 6.1[a]	92.0 ± 7.3[a]	4516.0 ± 241.2[c]	1774.0 ± 236.1[c]	972.5 ± 66.8[b]

Values that share different letter superscripts are significantly different ($P < 0.05$).

3.10 Antioxidant enzymes and Lipid peroxidation

In this study, there was a significant ($P < 0.05$) increase in CAT in the diabetic *K. pinnata* treated compared to the baseline high fat diet group. There was no significant ($P > 0.05$) difference in CAT activity in the control, diabetic control and the diabetic *K. pinnata* treated groups. There was a significant ($P < 0.05$) increase in the GSH levels in the diabetic *K. pinnata* treated group compared to the diabetic control group. It is important to note that there was a non-significant ($P > 0.05$) increase in GSH levels in the diabetic control group compared to the control group. There was a significant ($P < 0.05$) increase in SOD activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. It was noted that there was no significant ($P > 0.05$) difference in SOD activity of the diabetic *K. pinnata* treated and the diabetic Metformin group. TBARS showed that there was no significant ($P > 0.05$) difference in the levels of TBARS in the control and the diabetic *K. pinnata* treated group. It was noted that there was a non-significant ($P > 0.05$) decrease in TBARS levels in the diabetic *K. pinnata* treated and the diabetic control groups. (Table 5). Aqueous preparation of *K. pinnata* in diabetic rats compared to the diabetic control provided a non-significant ($P > 0.05$) increase in CAT, significant ($P < 0.05$) increase in GSH and SOD, and non-significant ($P > 0.05$) decrease in TBARS, these findings indicate that *K. pinnata* has antioxidant capabilities. Further studies must be conducted in order to establish the phytochemicals associated with this mechanism.

Table 5. Alteration of Antioxidant Indices in Type 2 Diabetic Rats Administered Aqueous Preparaton of *Kalanchoe pinnata*.

	CAT (mmol H2O2/min/mg protein)	GSH (uM/mg protein)	SOD (mU/mg protein/min)	TBARS (mmol MDA formed/mg protein)
Baseline Control	6.05 ± 2.02^{ab}	34.53 ± 8.58^{a}	12.67 ± 1.21^{a}	1.052 ± 0.05^{ab}
Baseline High fat diet	3.91 ± 0.13^{a}	51.32 ± 6.42^{ab}	13.43 ± 0.64^{a}	1.04 ± 0.05^{ab}
Baseline Diabetic	4.97 ± 0.22^{ab}	83.53 ± 9.45^{bc}	17.46 ± 0.82^{ab}	1.18 ± 0.04^{b}
Control	4.83 ± 0.98^{ab}	51.45 ± 13.01^{ab}	13.26 ± 0.80^{a}	1.05 ± 0.05^{ab}
High fat diet	3.69 ± 0.11^{ab}	59.61 ± 6.02^{ab}	16.24 ± 3.72^{ab}	0.96 ± 0.02^{a}
Diabetic Control	4.50 ± 0.32^{ab}	66.53 ± 8.52^{ab}	14.46 ± 0.95^{a}	1.17 ± 0.03^{b}
Diabetic *K. pinnata*	6.84 ± 1.02^{b}	101.81 ± 9.47^{c}	26.27 ± 5.69^{b}	1.09 ± 0.03^{ab}
Diabetic Metformin	5.01 ± 0.96^{ab}	65.59 ± 12.2^{ab}	18.43 ± 4.48^{ab}	1.14 ± 0.07^{b}

Values that share different letter superscripts are significantly different ($P < 0.05$). CAT= Catalase, GSH= Reduced Glutathione, SOD= Superoxide dismutase, TBARS= Thiobarbituric Acid Reactive Substances.

3.11 Enzymes of Lipid and Carbohydrate Metabolism in Liver and Kidney

Some metabolic enzyme activities in the liver and kidney of Type 2 diabetic rats were analyzed in this study. There was no significant ($P > 0.05$) difference in liver malic enzyme (ME) activity among the groups. There was a non-significant ($P > 0.05$) decrease in liver ME activity in the diabetic *K. pinnata* treated group compared to the diabetic control group, these results were indicative of aqueous preparation of *K. pinnata* potentially reducing lipogenesis of carbohydrates. There was also no significant ($P > 0.05$) difference in liver G6PD activity among the groups. There was a non-significant ($P > 0.05$) increase in liver G6PD activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. Hepatic pyruvate kinase activity in the liver indicated that there was a significant ($P < 0.05$) increase in the diabetic *K. pinnata* treated group compared to the diabetic control group (Table 6). Malic enzyme activity in the kidney indicated that there was no significant ($P > 0.05$) difference among the groups. However, there was a non-significant ($P > 0.05$) increase in renal ME activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. There was no significant ($P > 0.05$) difference in renal G6PD activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. There was a significant increase ($P < 0.05$) in renal G6PD activity in the baseline high fat diet group compared to the control. It was also noted that there was a significant increase ($P < 0.05$) renal G6PD activity in the diabetic Metformin treated group compared to the control group. This could be indicative of a normally functioning pentose phosphate pathway in the linking of glycolysis to lipogenesis, Figure 4 shows diabetic Metformin treated group significantly ($P < 0.05$)

gained weight in comparison to the diabetic *K. pinnata* treated group. There was a significant increase ($P < 0.05$) in liver PK activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. There was a non-significant ($P > 0.05$) increase in renal PK activity in the diabetic *K. pinnata* treated group compared to the diabetic control group. Aqueous preparation of *K. pinnata* could potentially increase the rate at which molecules progress through the glycolytic pathway with the increase of G6PD and PK in both the liver and the kidney and with an increase in renal ME. It was also noted that there was a significant decrease ($P < 0.05$) in PK activity in the diabetic Metformin treated group compared to the control group (Table 7). Further studies need to be conducted to establish the effect of lipid and carbohydrate metabolism on liver and kidney enzymes on Type 2 diabetic rats.

Table 6. Metabolic Enzyme Activities in the Liver of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata*.

	ME (µmole/min/mg protein)	G6PD (µmole/min/mg protein)	PK (µmole/min/mg protein)
Baseline Control	3.8E-05 ± 1.6E-05[a]	0.06 ± 0.006[a]	5.9E-05 ± 1.2E-05[ab]
Baseline High fat diet	4.5E-05 ± 1.7E-05[a]	0.07 ± 0.007[a]	2.9E-05 ± 7.6E-06[a]
Baseline Diabetic	2.9E-05 ± 9.5E-06[a]	0.05 ± 0.004[a]	4.6E-05 ± 9.0E-06[a]
Control	3.3E-05 ± 1.1E-05[a]	0.07 ± 0.005[a]	1.1E-04 ± 2.8E-05[b]
High fat diet	1.1E-05 ± 2.7E-06[a]	0.07 ± 0.009[a]	2.1E-05 ± 5.7E-06[a]
Diabetic Control	4.2E-05 ± 9.9E-06[a]	0.06 ± .0.003[a]	4.8E-05 ± 6.7E-06[a]
Diabetic *K. pinnata*	3.6E-05 ± 1.1E-05[a]	0.07 ± 0.006[a]	2.0E-04 ± 4.4E-05[c]
Diabetic Metformin	4.4E-05 ± 8.4E-06[a]	0.06 ± 0.007[a]	4.3E-05 ± 1.3E-05[a]

Values that share different letter superscripts are significantly different ($P < 0.05$). ME= Malic Enzyme, G6PD= Glucose-6-Phosphate Dehydrogenase, PK= Pyruvate Kinase.

Table 7. Metabolic Enzyme Activities in the Kidney of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata*.

	ME (µmole/min/mg protein)	G6PD (µmole/min/mg protein)	PK (µmole/min/mg protein)
Baseline Control	2.9E-05 ± 1.2E-05[a]	0.041 ± 0.0038[b]	4.1E-05 ± 4.2E-06[b]
Baseline High fat diet	4.3E-05 ± 1.8E-05[a]	0.042 ± 0.0019[b]	3.7E-05 ± 7.3E-06[b]
Baseline Diabetic	5.6E-05 ± 1.4E-05[a]	0.035 ± 0.0038[ab]	5.1E-05 ± 2.8E-06[b]
Control	3.7E-05 ± 1.3E-05[a]	0.031 ± 0.0015[a]	5.3E-05 ± 4.4E-06[b]
High fat diet	6.4E-05 ± 2.7E-05[a]	0.035 ± 0.0038[ab]	4.7E-05 ± 8.1E-06[b]
Diabetic Control	4.9E-05 ± 9E-06[a]	0.035 ± 0.0019[ab]	4.3E-05 ± 4.1E-06[b]
Diabetic *K. pinnata*	6.5E-05 ± 4.7E-06[a]	0.036 ± 0.0020[ab]	5.0E-05 ± 8.0E-06[b]
Diabetic Metformin	2.3E-05 ± 9.1E-06[a]	0.041 ± 0.0015[b]	1.7E-05 ± 1.2E-06[a]

Values that share different letter superscripts are significantly different ($P < 0.05$). ME= Malic Enzyme, G6PD= Glucose-6-Phosphate Dehydrogenase, PK= Pyruvate Kinase.

3.12 Inflammatory response

There was a non-significant ($P > 0.05$) decrease in serum IL-6 levels in the diabetic *K. pinnata* and diabetic Metformin treated groups compared to the diabetic control group (Figure 18). It was noted that there was no significant ($P > 0.05$) difference in IL-6 levels between baseline control, baseline high fat diet, baseline diabetic, control and diabetic control groups. There was a significant increase ($P < 0.05$) in IL-6 levels in the baseline control group compared to the high fat diet, diabetic *K. pinnata* and diabetic Metformin. Aqueous preparation of *K. pinnata* could potentially enhance insulin sensitivity in Type 2 diabetic patients by reducing IL-6 levels. Further studies are needed in order to confirm these findings.

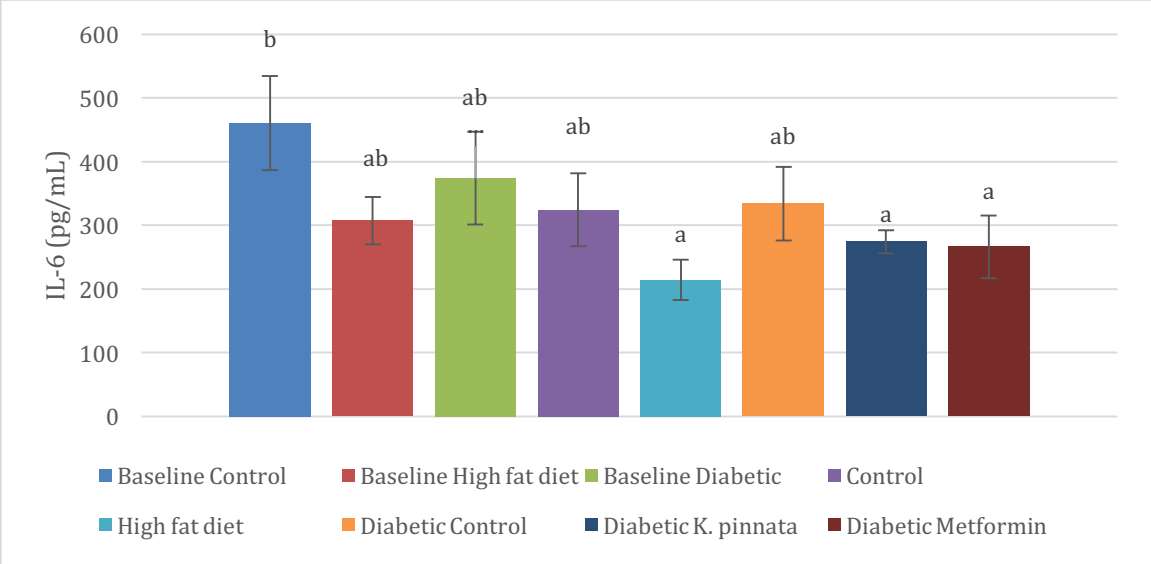

Figure 18. Interleukin-6 Levels in the Serum of Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata.* Figures that share different letter superscripts are significantly different ($P < 0.05$).

3.13 Summary of Results

Table 8.

Summary of Analytes in Type 2 Diabetic Rats Administered Aqueous Preparation of *Kalanchoe pinnata* Compared to the Diabetic Control Group

Fraction tested	Assay Conducted	Significance	Effect due to *K. pinnata* treatment compared to the diabetic control
	Glucose Level		
Blood	Glucose	Yes	Decrease
	Serum Protein Profile		
Serum	Albumin	No	Decrease
Serum	Globulin	No	Increase
Serum	Total Protein	No	Increase
Serum	Albumin:Globulin Ratio	No	Increase
	Lipid Profile		
Serum	Triglycerides	Yes	Decrease
Serum	Total Cholesterol	No	Increase
Serum	HDL-Cholesterol	Yes	Increase
	Effect on Oxidative Stress		
Serum	Catalase	No	Increase
Serum	Reduced Glutathione	Yes	Increase
Serum	Superoxide Dismutase	Yes	Increase
Serum	Thiobarbituric acid reactive substances	No	Decrease
	Digestive Enzymes		
Serum	Amylase	No	Increase
Proximal Intestine	Amylase	No	Decrease
Distal Intestine	Amylase	No	Decrease
	Metabolic Enzymes		
Liver	Pyruvate Kinase	No	Increase
Kidney	Pyruvate Kinase	No	Increase
Liver	Malic Enzyme	No	Decrease
Kidney	Malic Enzyme	No	Increase

Table 8. Continued.

Liver	Glucose-6-Phosphate Dehydrogenase	No	Increase
Kidney	Glucose-6-Phosphate Dehydrogenase	No	Increase
	Liver Function Enzymes		
Serum	Alanine Amino Transferase	Yes	Increase
Serum	Aspartate Amino Transferase	No	Decrease
Serum	Alanine Phosphatase	Yes	Increase
	Kidney Function Indices		
Serum	Uric Acid	Yes	Increase
Serum	Blood Urea Nitrogen	No	Increase
Serum	Creatinine	No	Increase
	Complete Blood Count		
Total Blood	Basophils	No	Increase
Total Blood	Eosinohils	Yes	Decrease
Total Blood	Lymphocytes	No	Increase
Total Blood	Monocytes	No	Increase
Total Blood	Neutrophils	Yes	Decrease
Total Blood	White Blood Cells	Yes	Increase
Total Blood	Red Blood Cells	No	Increase
Total Blood	Hemoglobin	Yes	Increase
	Inflammatory Response		
Serum	Interleukin-6	No	Decrease
	Serum Leptin and Insulin		
Serum	Leptin	No	Increase
Serum	Insulin	No	Increase
	Serum Electrolytes		
Serum	Phosphorus	No	Decrease
Serum	Calcium	No	Increase

4. Conclusion

Due to the prevalence of Type 2 diabetes it is important to find a cost effective alternative in order to combat this disease. The issue with the use of medicinal plant alternatives is the lack of scientific research and data to support its use. This study evaluated the use of aqueous *K. pinnata,* extract preparation consumption in the treatment of Type 2 diabetes using animal models.

Our findings indicated that the aqueous preparation of *K. pinnata* may be beneficial in the treatment of Type 2 diabetes. Aqueous preparation of *K. pinnata* for Type 2 diabetic rats provided a significant amount of weight loss and the significant reduction of blood glucose through the enhancement of insulin secretion. *Kalanchoe pinnata* treated group lost significantly more weight than the diabetic Metformin treated group. Blood glucose levels for the diabetic Metformin and diabetic *K. pinnata* treated groups were not significantly different. Aqueous preparation of *K. pinnata* in Type 2 diabetic rats also increased the production of amylase due to the potential restoration of insulin. The decrease in albumin, globulin and total proteins could be due to the hypoglycemic effects of aqueous preparation of *K. pinnata* and Metformin in Type 2 diabetic rats. Aqueous preparation of *K. pinnata* may allow the metabolism of lipids due to insulin restoration and may repair phosphorus metabolism because of hypoglycemic abilities. Increased activities of ALP and ALT could be indicative of the adverse effects of the aqueous preparation of *K. pinnata* on the liver. Diabetic Metformin treated group also showed a significant increase in ALP levels, which could indicate liver damage. Increased levels of uric acid due to aqueous preparation of *K. pinnata* could affect kidney function. Aqueous

preparation of *K. pinnata* and Metformin was shown to increase the production of leptin and insulin. Leptin aided in weight reduction, and insulin secretion potentially repaired the beta-cells of the islets within the pancreas of the rats. Treatment with *K. pinnata* and Metformin increased RBC and HGB levels to potentially compensate for the pathology of the disease by increasing blood oxygen supplies to the pancreas and attempt to maintain renal and microvascular function. Restoration in glycemic control can be seen with the treatment of aqueous preparation of *K. pinnata* due to the increase in basophils, lymphocytes and significant decrease in neutrophils. Aqueous preparation of *K. pinnata* provided antioxidant capabilities in the treatment of Type 2 diabetic rats. *Kalanchoe pinnata* can potentially reduce lipogenesis through a decrease in the activities of liver ME. Increased renal G6PD activities link glycolysis to lipogenesis through a normally functioning pentose phosphate pathway. Increased PK activities in both the liver and kidney could potentially increase the rate at which molecules are processed in the glycolytic pathway. Our results also confirmed that aqueous preparation of *K. pinnata* could potentially enhance insulin sensitivity in Type 2 diabetes. Further investigation is needed in order to establish the benefits and adverse effects of aqueous preparation of *K. pinnata*. Future studies include combining *Kalanchoe pinnata* with Metformin in order to determine the benefits and adverse effects for the treatment of Type 2 diabetes.

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