EFFECTS OF DIETARY SUPPLEMENTATION OF *Persea americana* (AVOCADO) POLYPHENOLIC PEEL EXTRACT ON ALLOXAN-INDUCED DIABETIC DYSLIPIDAEMIA AND OXIDATIVE STRESS IN MALE WISTAR RATS

BY

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DECLARATION

I, MATTHEW, EBOSEREMEN IYOBHEBHE, a M.Sc. student in the Department of Biochemistry, Landmark University, Omu-Aran, hereby declare that this thesis entitled "Effects of *Persea americana* peel polyphenolic extract on alloxan-induced dyslipidemia and oxidative stress in male Wistar rats", submitted by me is based on my original work. Any material(s) obtained from other sources or work done by any other persons or institutions have been duly acknowledged.

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CERTIFICATION

This is to certify that this thesis has been read and approved as meeting the requirements of the Department of Biochemistry, Landmark University, Omu-Aran, Nigeria, for the award of M.Sc.

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ABSTRACT

The increment in the cost as well as side effects of synthetic drugs has drawn the attention to the exploration of health-promoting attributes of plants in our diets. This present study sought to evaluate the effects of dietary supplementation of avocado pear peel polyphenolic extract complications resulting from alloxan-induced diabetes in rats. Diabetes was induced in the experimental animals by a single intraperitoneal injection of 150 mg/kg body weight (b.w) alloxan. Thirty (30) male diabetic rats (287.54 ± 6.02g) were randomly assigned into five groups (n = 6) consisting diabetic control (DC), 50 mg/kg, 100 mg/kg, and 200 mg/kg avocado polyphenolic peel extract, and metformin. A separate group of six non-diabetic rats served as negative control (NC). Animals in NC, DC and metformin groups were fed normal rat pellets while 50 mg/kg, 100 mg/kg, and 200 mg/kg avocado polyphenolic peel extract supplementation were fed normal diet supplemented with 50, 100 and 200 mg/kg avocado polyphenolic peel extract respectively. Diet and water were given *ad libitum* for a period of 21 days while monitoring body weight and blood glucose weekly. Following the feeding experiment, a significant ($p < 0.05$) reduction in blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and malondialdehyde (MDA) concentrations were observed in diabetic rats fed avocado polyphenolic peel extract-supplemented diets compared to DC. Serum insulin concentration as well as the calculated homoestatic model assessment (HOMA-β) score were significantly higher as opposed to reduced homoestatic model assessment insulin resistance (HOMA-IR) score in rats fed avocado polyphenolic peel extract-supplemented diets compared to DC. Serum antioxidant enzymes (catalase and superoxide dismutase) activities and glutathione concentration were remarkably enhanced in rats fed avocado polyphenolic peel extract-supplemented diets compared to DC. Based on the results generated from this study, it could be concluded that dietary avocado polyphenolic peel

extract-supplementation abrogates diabetic induced dyslipidaemia and oxidative stress in rats. The isolation of the beneficial compound found in the polyphenolic extract of the peel, as well as its application in pharmaceutical and food industries could be as well looked into further.

DEDICATION

This thesis is dedicated to my father, my first mentor, my hero, a man I love so much, Late Pstr. F.O. Iyobhebhe who raised me as a child, and put me in the path I am today who was also my inspiration who believed in me and sent me to school; To my loving and caring mother, Mrs. V.A. Iyobhebhe who brought me into this world and mean a lot to me, for your prayers, love that you showed me, the support you gave me, and encouragement; To my lovely siblings who gave me their support all through this programme.

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life as a person regarding this programme which led to the huge success of this work. I owe you all a great deal of gratitude.

Iyobhebhe, M.E.

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CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic syndrome that has increased over the past few decades (World Health Organization, 2016). The global prevalence of DM has risen to 8.5% amounting approximately 422 million in 2014 (World Health Organization, 2016). It has been predicted that, the incidence of this syndrome would be more than double by the year of 2030 (Fernández-Millán, Ramos, Alvarez, Bravo, Goya *et al*., 2014). Amongst all diagnosed cases, type 2 diabetes mellitus (T2DM) is more rampant and comprising approximately 90−95% of total diabetic cases (Khanra, Dewanjee, Dua, Sahu, Gangopadhyay *et al*., 2015). Hyperglycemia arbitrated glucose toxicity resulting in a number of homeostatic disturbances within the organs, which leads to a number of complications.

Over the years, many synthetic antidiabetic agents have been developed to control hyperglycemia and some include insulin, thiazolidinediones, glucagon-like peptide-1 receptor agonist, α-glucosidase inhibitors, sodium-glucose cotransporter type-2 inhibitors, sulfonylureas, dipeptidyl peptidase 4 inhibitors, and biguanides (Yaribeygi, Panahi, Javadi, Sahebkar, 2018). However, they may have limited efficacy or unfavorable side effects including hypoglycemic coma and disturbances of the liver and kidneys (Chaudhury, Duvoor, Reddy Dendi, Kraleti, Chada, *et al*., 2017). This presents an adjunct treatment option from herbal-based agents against diabetes (Yaribeygi *et al*., 2019) as they are easily available, generally lower in cost, and said to be less toxic when compared to synthetic antidiabetic agents (Eddouks, Bidi, El Bouhali, Hajji, and Zeggwagh, *et al*., 2014; Kaur, Garg, Gulati, and Kumar Singh, *et al*., 2016).

Avocado (*Persea americana*) which originated in Mexico and belongs to the *Lauraceae* family, is now been planted and grown in tropical and sub-tropical regions throughout the world. The peel which has been the most under-utilized part of the fruit is reported to contain high phenolic content and antioxidant activity. Besides this, the peel has been demonstrated to show effectual antimicrobial, antibiotic and anti-inflammatory properties (Adikaram, Ewing, Karunaratne, and Wijeratne *et al*., 1992). Within this scope, it is a promising material for the production of functional foods and pharmaceutical products, and also it can be used as a bio-source for the production of environment friendly adsorbents (Antasionasti, Riyanto, and Rohman *et al*., 2017; Palma, Lloret, Puen, Tobar, and Contreras, *et al*., 2016).

1.1 STATEMENT OF PROBLEM

According to Lipinski (2001), elevated level of reactive oxygen species (ROS in diabetes may be due to a decrease in destruction or an increase in the production by catalase, superoxide dismutase and glutathione peroxidase antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications. Many evidences from research works have given link between diabetes and oxidative stress by measuring various biomarkers that include DNA damage biomarkers and lipid peroxidation products. It is believed that in the onset and progression of late diabetic complication, free radicals have got a major role due to their ability to damage lipids, proteins and DNA (Ayepola, Brooks, and Oguntibeju, 2014).

Persistent hyperglycemia introduces toxic effects through a sequence of secondary transducers which bring about the excess generation of reactive oxygen species (ROS) in the myocardial tissue via activation of polyol pathway a pathway, production of excess advanced glycation end-products (AGEs) which leads to myocardial inflammation, collagen deposition and fibrosis (Riaz, Zia-Ul-Haq, and Saad, *et al*., 2016).

Therefore, the search for more effective and safer antidiabetic agents continues to be an important area for research, following the recommendations from the World Health Organization on the beneficial uses of medicinal plants in the management/treatment of diabetes mellitus (Mamdouh, Mikhailov, and Muller *et al.,* 2009).

1.2 JUSTIFICATION OF THE STUDY

In 2019, it was estimated that 463 million people (95% confidence interval: 369–601 million) are living with diabetes which has led to the question of why is there a continuous increase in this disease compared to the years before. However, attempts have been made in combating this disease through different means which include; either by production of synthetic drugs, or the traditional use of plants.

In recent years, the role played by omega 3 polyunsaturated fatty acid (PUFA) in promoting health benefit has been the subject of an increasing number of studies (Lalia and Lanza, 2016). Studies in rodents and humans have indicated that omega 3 PUFA potentially elicit effects which might be useful for reducing obesity and related metabolic disease, energy expenditure and reduced fat deposition (Buckley and Howe, 2010).

Given that half a billion people are living with diabetes, there is an urgent need for developing and implementing multi-sectoral strategies to tackle diabetes. Without urgent and sufficient actions, it is predicted that 578 million people will have diabetes by 2030 and the number will increase by 51% (700 million) in 2045 (Saeedi, Petersohn, Salpea, Malanda, Karuranga, *et al,* 2019).

One of the means of contributing towards tackling this issue is through research from easily available, cost effective, safer, and easily accessible natural materials. Therefore, this study investigates the effects of avocado polyphenolic peel extract supplementation as a potential anti-diabetic agent.

1.3 AIM

The aim of this study is to evaluate the potential effect of *P. americana* polyphenolic peel extract dietary supplementation on alloxan-induced dyslipidemia and oxidative stress in male Wistar rats.

1.4 OBJECTIVES

The following are the objectives of the current study;

- To investigate the effect of avocado polyphenolic peel extract-supplementation on blood glucose level following the induction of diabetes in male rats.
- To investigate the effect of avocado polyphenolic peel extract-supplementation on selected antioxidant markers following the induction of diabetes in male rats.
- To investigate the effect of avocado polyphenolic peel extract-supplementation on serum lipid profiles following the induction of diabetes in male rats.

- To investigate the impact of avocado polyphenolic peel extract-supplementation on regulatory mechanisms following the induction of diabetes in male rats.

1.5 RESEARCH QUESTIONS

- Will avocado polyphenolic peel extractsupplementation be able to prevent complications arising from metabolic derangement in Diabetes mellitus?

- Will avocado polyphenolic peel extractsupplementation be effective in the management of Diabetes mellitus?

CHAPTER TWO

2.0 Literature review

2.1 Diabetes mellitus

2.1.1 Pathogenesis of type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) represents up to 10% of the cases found to be diabetes and one major mark is its occurrence in the early stages of life. It majorly arises from the destruction of the β cells of the pancreas. The percentage of affected individual which is below 10% are classified as a type 1B because there has been no evidence of any autoimmunity as well as the origin of such cases been idiopathic (Stavroula, Nektaria, Papadopoulous, George and Christina 2018). Only 10–15% of the patients have either a relative that has a record of this disease. However, the lifetime implication of developing type 1 diabetes is greatly increased in the relatives of the individual with the disease with statistics that the children are 6%, siblings like 5% and in the situation of twins about 50% is predisposed to the disease compared to the general population prevalence of 0.4% (Beyan, Riese, Hawa, Beretta, Davidson et al., 2012)

2.1.2 Pathogenesis of type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a complex ailment comprising of different factors such as environmental factors, epigenetics, lifestyle, and genetics, mostly high calory diets (Schulze and Hu 2005). A major signal of T2DM includes high level of blood sugar concentration and increased level of lipids in the blood (dyslipidemia) and these attributes are contributing factors in the production of free radicals that causes oxidative stress (Erukainure, Ijomone, Oyebode, Chukwuma, Aschner *et al.,* 2019). Receptive oxygen species/RNS-initiated oxidative pressure influences ordinary cell digestion of carbs, proteins, fats and electrolytes,

prompting genome and epigenome unsteadiness, cell harm, irritation and hinder the responsibility of the organ (Jiang, Briedé, Jennen, Van Summeren, Saritas-Brauers, *et al.,* 2015). One of the distinctive features of pancreatic β-cells specifically includes low endogenous antioxidant capacity, which gives them the ability to be prone to oxidative stress (Wang and Wang, 2017).

2.2 Prevalence of diabetes mellitus

Diabetes mellitus over the past few years have been known to be one of the most common and killer diseases today, and by so doing the International Diabetes Federation (IDF) has put in place statistical data and in order to monitor the percentage increase of this disease and show below in the table the prevalence of diabetes mellitus.

Table 1: Prevalence of diabetes mellitus in the World, Africa and Nigeria

Selected IDF Regions	2019	2045	Percentage Increase (%)
World	463,000,000	700,200,000	21.1
Africa	19,400,000	28,600,000	11.1
Nigeria	2,700,000	5,400,000	16

Source: Adapted from IDF. (2019)

2.3 Classes of Diabetes mellitus

2.3.1 Type-1 Diabetes mellitus

Type-1 diabetes mellitus or insulin-dependent diabetes mellitus (IDDM), is a chronic disorder in which the pancreas produces little or no insulin (Nokoff and Rewers, 2013). The specific destruction of the pancreatic β-cells is the leading and major cause of this type of diabetes and it is related to immune-mediated damage (Zhao, 2011). This type of diabetes is common to all the age groups beginning

from the age of 4 years old also, about 10% of individuals are living with diabetes. (Nokoff and Rewers, 2013). When the body fail to utilize the secreted insulin produced in the beta-cell of the pancreas, which will lead to the breakdown of fat deposits in the body in order to produce the energy to replace the depleted energy in a process called diabetic ketoacidosis (Ayepola, Brooks, and Oguntibeju, *et al*., 2014a).

2.3.2 Type 2 Diabetes mellitus

Type-2 diabetes is a heterogeneous disorder portrayed by insulin obstruction followed by impeded insulin emission by β-cells in the pancreas (Sears and Perry, 2015). The primary imperfections in the beginning of hyperglycemia in T2D incorporates expanded hepatic glucose creation, diminished insulin emission and hindered insulin activity/insulin opposition (DeFronzo, Ferrannini, Groop, Henry, Herman *et al*., 2015).

Blood insulin levels are raised, and β-cell work is mostly inhibited. In stage two, the disease advances to Type-2 diabetes because there is little or no work from the β-cell (American Diabetes Association, 2018).

2.3.3 Gestational diabetes

Gestational diabetes mellitus (GDM) is another kind of diabetes and it is analyzed during pregnancy (Rani and Begum, 2016). It is mostly common amongst the African Americans, Latino Americans and American Indians (Hedderson, Gunderson, and Ferrara, 2010). Gestational diabetes expects treatment to standardize maternal blood glucose level to keep away from complexities in the newborn child (Saravanan, Magee, Banerjee, Coleman, Von Dadelszen, Denison,

et al., 2020). Much of the time, gestational diabetes stops after birth (Tierney and rancher, 2002).

2.4 Symptoms of Diabetes mellitus

Indications of diabetes mellitus are polydipsia (successive thirst), polyuria (over the top pee) and polyphagia (unreasonable yearning or craving) (Roglic, 2016). These manifestations foster rapidly in type 1 diabetes mellitus and all the more leisurely or totally missing in type 2 diabetes mellitus (American Diabetes Association, 2018).

Manifestations like stomach torment, decreased craving, queasiness and spewing could likewise avocado polyphenolic peel extract. Different manifestations incorporate unexplained weight reduction, obscured vision, repetitive diseases, including skin contaminations, gum disease, and urinary plot diseases, slow mending of wounds, skin issues, exhaustion, and deadness, shivering or torment in the feet, legs or hands (Roglic, 2016).

2.5 Complications of Diabetes mellitus

2.5.1 Macrovascular Complications

Macrovascular difficulties of diabetes are fundamentally sicknesses of the coronary veins, fringe corridors, and cerebrovascular (Nair, 2007). Cardiovascular problems represent the best part of medical care consumptions in individuals with diabetes mellitus (Patterson, Holt, Nienaber, Fairman, Heijmen, et al., 2014). Full scale vascular entanglements coming about because of diabetes mellitus are examined underneath.

2.5.1.1 Diabetic Cardiomyopathy

Diabetic cardiomyopathy clarifies diabetes-related changes in the construction and capacity of the myocardium that isn't straightforwardly inferable from other factors like coronary vein illness (CAD) or hypertension. Atherosclerosis, (extreme collection of lipids, cholesterol, incendiary cells, and connective tissue in the vessel divider) represents over 80% of the cardiovascular illness related with death and incapacity (Avogaro, de Kreutzenberg, Negut, Tiengo, and Scognamiglio, 2004; Rerkpattanapipat, D'Agostino, Link, Shahar, Lima, et al., 2009). Development of atherosclerotic plaques prompts obstacle of vessel lumen and a quick discontinuance in blood stream to target tissue (Funk, Yurdagul, and Orr, 2012).

2.5.2 Microvascular complications

Intracellular hyperglycaemia just as hereditary inclination and in the end influence the microvasculature, prompting inconveniences mostly from the kidneys, the eyes and the sensory system (Faselis, Katsimardou, Imprialos, Deligkaris, Kallistratos, et al., 2020). Microvascular complexities are obligatory, as it is assessed that up to 25% of recently determined patients to have T2DM have as of now created at least one confusions of DM.

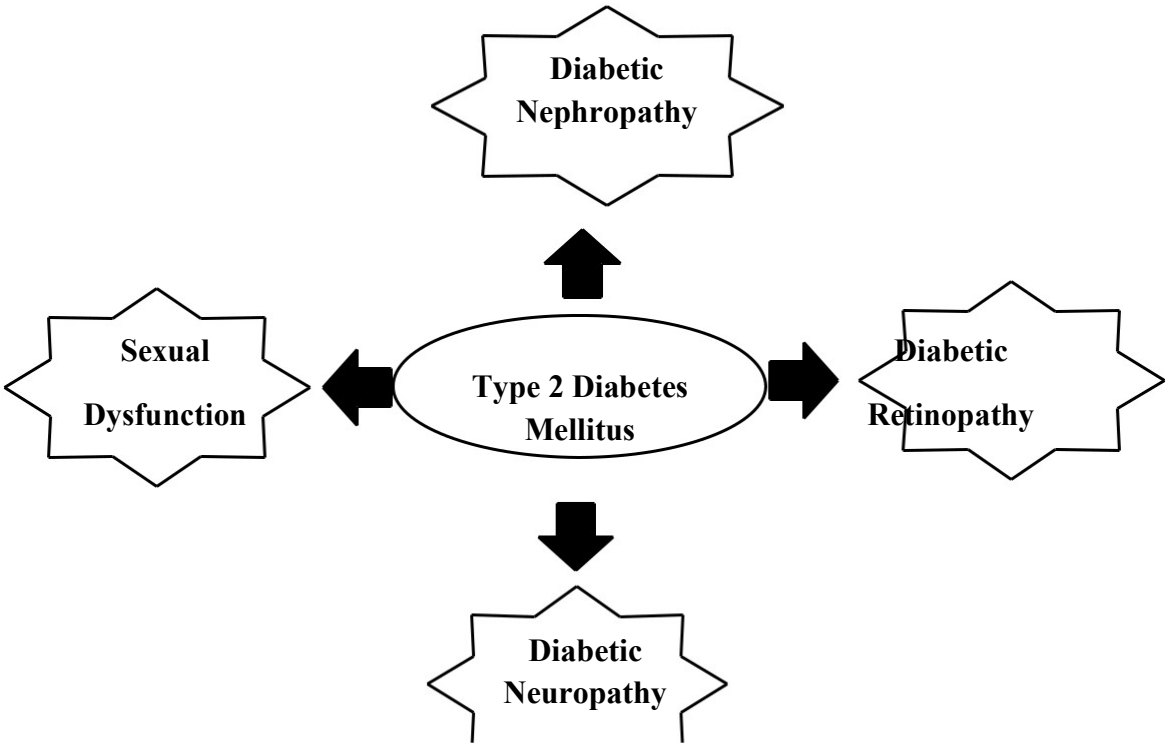

Figure 1: Complications arising from Type 2 Diabetes Mellitus

Source: (Faselis, Katsimardou, Imprialos, Deligkaris, Kallistratos, et al., 2020**).**

2.5.2.1 Diabetic Nephropathy

Diabetic kidney illness (DKD) is a typical microvascular difficulty of DM, influencing roughly 25% of the diabetic populace (Zelnick, Weiss, Kestenbaum, Robinson-Cohen, Heagerty, et al., 2017). Besides, DM is the significant reason for ESRD in the created world, representing half, everything being equal (Tuttle, Bakris, Bilous, Chiang, De Boer, et al., 2014). In the meantime, there is a set up connection among albuminuria and cardiovascular infection (CVD). In particular, microalbuminuria is considered as a danger factor for CVD, while intercessions to bring down albuminuria positively affect cardiovascular security (de Zeeuw, Parving, and Henning, 2006).

2.6.2.2 Diabetic Neuropathy

Diabetic neuropathy is a nerve brokenness that can havocado polyphenolic peel extractn in people with diabetes mellitus (American Diabetes Association, 2018). High glucose can harm nerve filaments of the entire body, however diabetic neuropathy most occasions harm nerves in the legs and feet. In view of the influenced nerves, indications of diabetic neuropathy can prompt tangible misfortune, injury to the avocado polyphenolic peel extractndages and removal of the lower limits (Pazdro and Burgess, 2010; Hosseini-Zare, Dashti-Khavidaki, Mahdavi-Mazdeh, Ahmadi, and Akrami, 2012; Javed, Jumean, Murad, Okorodudu, Kumar, et al., 2015). Various metabolic elements have been involved in the advancement of type-2 diabetes related neuropathy. These incorporate collection of non-enzymatic progressed glycated finished results (AGE) on nerve and actuation of intracellular flagging atoms like protein kinase C (PKC) (Pop-Busui, Ang, Holmes, Gallagher, and Feldman, et al., 2016).

Diminished perspiring, shivering, consuming sensation, deadness, loss of reflexes Signs, shortcoming are essential side effects of diabetic neuropathy (Griebeler, Morey-Vargas, Brito, Tsapas, Wang, et al., 2014). Eye floaters, spots, obscured vision, twofold vision, and eye torment are altogether indications of diabetic retinopathy (Solomon, Chew, Duh, Sobrin, Sun, et al., 2017).

2.5.2.3 Diabetic Retinopathy

Diabetic retinopathy alludes to the harm caused to the capacity of the retina and the macula, which prompts the incomplete or even entire visual impairment incapacity in patients with T2D. (Wong and Heriot, (2008). It is a perceived result

of severely managed diabetes mellitus which causes momentous loss of vision and visual impairment (Beltramo and Porta, 2013). Diabetic retinopathy predominantly influence the vascular parts of the retina (Shin, Sorenson, and Sheibani, 2014). Eye floaters, spots, obscured vision, twofold vision, and eye torment are altogether indications of diabetic retinopathy (Solomon, Chew, Duh, Sobrin, Sun, et al., 2017).

2.5.2.4 Sexual Dysfunction

Sexual dysfunction in T2DM patients is a difficulty that has been generally over-looked in time past, in spite of the great effect of this condition on personal satisfaction. The pathogenesis of erectile brokenness (ED) in diabetic patients is extremely complicated and it is a combination of vasculopathic, neuropathic and hormonal changes that are credited to DM. It is an indication of microangiopathy, autonomic neuropathy and macroangiopathy and accordingly, ED could be taken advantage of as an early biomarker for diabetic complexities empowering early intercession and better results. ED is characterized as the determined or repetitive powerlessness to achieve as well as keep a penile erection adequate for effective sex (Consensus, 1993).

2.6 Tests for Diabetes mellitus

2.6.1 Blood test

Several blood tests used regularly in the diagnosis of diabetes mellitus include the following:

2.6.1.1 Fasting blood glucose test

The level of the fasting blood glucose is relative to the severity of diabetes mellitus. The test is regularly done in the early hours of the morning; the patient is relied upon to quick from nourishment for no less than eight hours before the test. A spot of the blood is taken from the patient's thumb. A plasma level of 200 mg/dl or more, significantly means that diabetes mellitus given medications like glucocorticoids are not properly managed (American Diabetes Association, 2018).

2.6.1.2 Oral glucose tolerance test

Oral glucose resilience test assesses the freedom of glucose from the blood after a characterized glucose load (Ngugi, Njagi, Kibiti, Ngeranwa, and Njagi, et al., 2012). The people quick for 8-14 hours, the blood test is removed and tried for fasting blood glucose level, glucose arrangement is given from there on, and blood is removed at stretches for the assurance of glucose (Bonora and Tuomilehto, 2011). The main example for simple diabetes screening is the one acquired 2 hours after glucose load. A plasma glucose level of 200 mg/dl or higher following two hours of glucose load affirms diabetic state (American Diabetes Association, 2018).

2.6.2 Urine test

Dipstick tests are utilized for the discovery of ketones in the pee (Baris, Sever, Aksay, Dogan, Yalcin, et al., 2014). Pee test are attempted to break down ketone bodies, glucose and proteins in the pee. The particular gravity of typical pee test is between the scopes of 1.002 to 1.020 (Baris, Sever, Aksay, Dogan, Yalcin, et al., 2014). Clinistix and Diastix are paper strips utilized for the conclusion of

glucose level in the pee. The test strip shading change is contrasted with an outline that shows the measure of glucose in the pee.

2.7 Treatment for Diabetes mellitus

Diabetes mellitus treatment is solely to rescue and revive health as well as to alleviate symptoms.

2.7.1 Anti-Diabetic drugs

Anti-diabetic medications are utilized in improving metabolic inconveniences that arises from diabetes mellitus. These medications are normally managed in combination with the nutritional requirement of the body. Oral enemy of diabetic medications are ordered into three categories. Medications in this category has to do with increase in the endogenous accessibility of the insulin and they includes; sulphonylureas for instance glibenclamide, the glinides, insulin analogs, glucagon-like peptide 1 (GLP-1 agonist and dipeptidyl peptidase-IV (DPP-IV). The other category of drug act against diabetes and tend to improve the effect of insulin, for instance thiazolidinediones, which are agonist of the peroxisome proliferator enacted receptor gamma (PPARγ) and the biguanide metformin. The third category comprises of α-amylase and α-glucosidase inhibitors for instance acarbose, which decrease the assimilation of polysaccharides (Ezuruike and Prieto, 2014).

2.7.2 Diet

Around 20-50% half of diabetic patients can handle their blood glucose levels by dietary alteration alone (World Health Organization, 2004). The creation of diet can diminish the movement of entanglements identified with the type 2

diabetes mellitus. (Eleazu, 2016). High amylose starch has a capability of controlling high-fat eating routine incited weight by regulating hepatic unsaturated fat oxidation (Shimotoyodome, Suzuki, Fukuoka, Tokimitsu, and Hase, 2010).

2.7.3 Insulin

Insulin is a polypeptide chemical hormone in the β-cells of the islets of Langerhans in the pancreas. It is made of two amino chains; A (acidic) and B (essential) combined by disulphide bond. The islets establish a piece of the pancreas, which represents 2% of its mass and β-cells comprise 60-80% of the cells of the Langerhans (Quesada, Tudurí, Ripoll, and Nadal, 2008). Insulin is corrupted in the gastrointestinal lot; thus, insulin can't be managed orally yet subcutaneously much of the time (Ballington and Laughlin, 2010). The major physiological job of insulin is to advance the blend of carb, protein, lipid and cores corrosive. Insulin impacts on sugar digestion incorporate guideline of hepatic glycogen blend, restraint of glycogenolysis, hindrance of gluconeogenesis, incitement of glucose transport across the muscle and adipocyte (Dimitriadis, Mitrou, Lambadiari, Boutati, Maratou, et al., 2011).

2.8 Alloxan

Alloxan; a volatile flexible compound, (Lenzen and munday, 1991) having its chemical structure like that of glucose (Figure 2) (Gorus, Malaisse, and Pipeleers, 1982). The hydrophilic nature of both Alloxan and glucose signifies that it is impossible for them to pass through the lipid bilayer of the plasma film. Basically, alloxan is the same as glucose, to the extent that the glucose transporter 2 (GLUT2) present in the plasma film of the β-cell carries it into the cytosol where it begins to cause damages to the cells (Gorus, Malaisse, and Pipeleers, 1982; Weaver, Mc

Daniel, and Lacy, 1978). The ability of the transporter is not by any means subjected by the impact of Alloxan (Elsner, Tiedge, Guldbakke, Munday, and Lenzen, 2002), which then, at that point makes it simpler and easier for it to enter into the β-cell without any restriction necessarily (Boquist, Nelson, Lorentzon, 1983; Malaisse, Doherty, Ladriere, Malaisse-Lagae, 2001). Thus, the poisonous impact of the Alloxan is not transferred to the cells producing insulin provided they have no impact with the GLUT2 (Elsner, Tiedge, Guldbakke, Munday, and Lenzen, 2002; Bloch, Zemel, Bloch, Grief, Vardi, 2000).

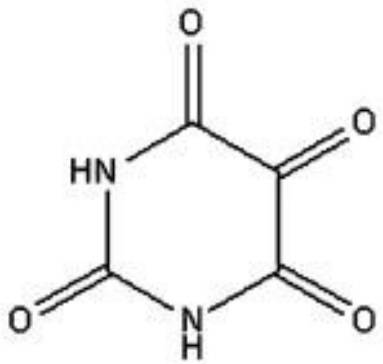

Figure 2: Alloxan

Source: (Macdonald, Ighodaro, Adeosun, and Akinloye, 2017)

2.8.1 Mechanism of Action

Alloxan has two different pathogenic effects: it selectively reduces glucose-induced insulin production by inhibiting glucokinase, the beta cell's glucose sensor, and it induces insulin-dependent diabetes by inducing ROS generation, culminating in beta cell death. These two effects can be attributed to alloxan's chemical characteristics, with the common denominator being the beta cell's selective absorption and accumulation of alloxan.

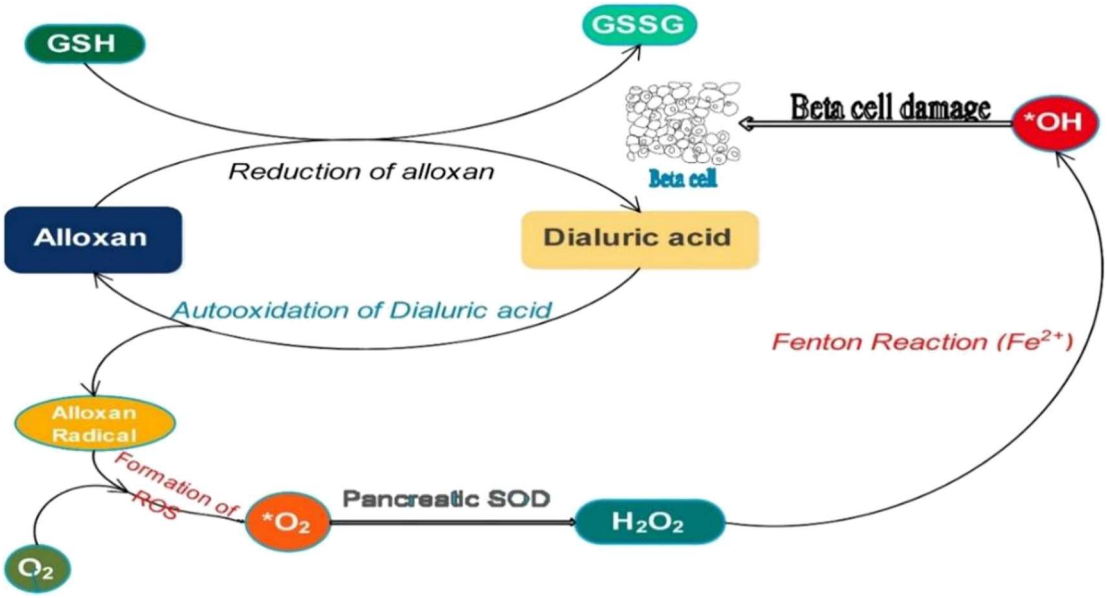

Figure 3: Alloxan Mechanism of action

Source: (Brömme, Mörke, Peschke, Ebelt, 2000**)**

In a cyclic reaction with its reduction product, dialuric acid (Figure 3), alloxan can create reactive oxygen species (ROS) (Cohen and Heikkila 1974; Munday. (1988); Winterbourn, Cowden, Sutton, 1989). Chemical redox cycling interactions between alloxan and dialuric acid, as well as cytoprotective enzymes' protective effects. Alloxan's harmful effect in beta cells is triggered by free radicals generated during this redox process (Oberley, 1988; Brömme, Mörke, Peschke, Ebelt, 2000). Autoxidation of dialuric corrosive produces superoxide extremists and hydrogen peroxide, and in the Fenton response, within the sight of an appropriate metal impetus (commonly iron), hydroxyl revolutionaries. The autoxidation of dialuric corrosive includes the middle of the road arrangement of the alloxan revolutionary (Winterbourn, Cowden, Sutton, 1989; Winterbourn and Munday, 1989

2.10 Oxidative stress

Oxidative pressure is a fundamental metabolic anomaly engaged with the improvement of diabetic inconveniences (Xu and Zou, 2009). It results when there is an expanded creation of responsive oxygen species past the limit of cell reinforcement framework. ROS creation could lead to the increase in the digestion rate of glucose, free unsaturated fat and other responsive metabolites in the diabetic mellitus state (Scott and King, 2004). The organs that are significantly influenced by expanded responsive oxygen species creation in diabetes mellitus incorporate liver, kidney, endothelia cells and pancreas (Lee, Seo, and Jiang, 2005).

2.10.1 The role of the Polyol pathway in oxidative stress

Increased production of sorbitol and fructose through the polyol pathway is another suggested route for DM-induced oxidative stress (Lee, Chung, and Chung, 1995). Activation of this system avocado polyphenolic peel extractors to be aided by increased ROS, but it has also been observed that activation of this system leads to DM-induced oxidative stress (Evans, Goldfine, Maddux, and Grodsky, 2002). Aldose reductase (AR), the first enzyme in this system, is a cytosolic enzyme that uses NADPH as a cofactor to convert glucose to sorbitol. The second enzyme, sorbitol dehydrogenase (SDH), transforms sorbitol to fructose with the help of its cofactor NAD+ (Wilson, Bohren, Gabbay, and Quiocho, 1992). The discovery that activation of the polyol process by high glucose burns NADPH, which is required for rebuilding the essential intracellular antioxidant reduced GSH, is one hypothesized explanation for the polyol system's contribution to oxidative stress.

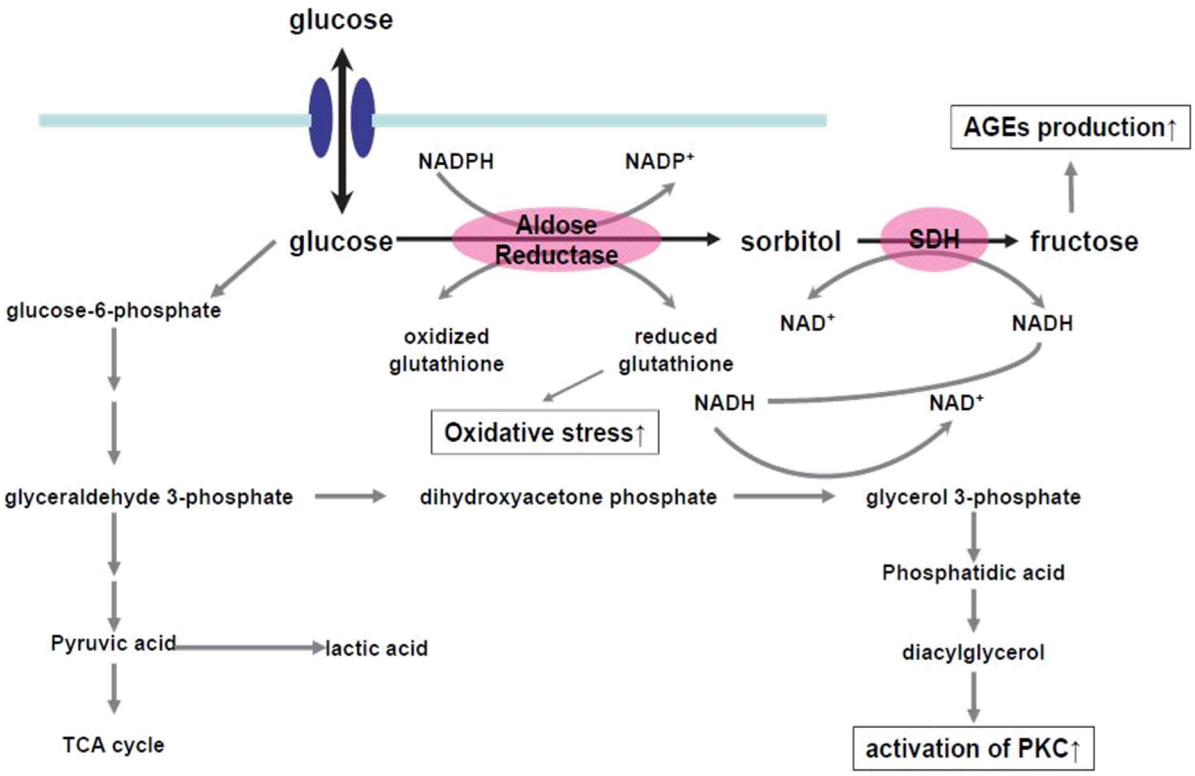

Figure 4: Role of the polyol pathway on vascular damage in diabetes

Source: Katakami (2018)

2.10.2 Glucose Autoxidation and formation of advanced glycation end products (AGEs)

Glucose autoxidation and the development of AGE, as referenced above, address a significant wellspring of DM-incited oxidative pressure. It has been recently announced that glucose can go through autoxidation under physiologic conditions creating protein responsive ketoaldehyde (dicarbonyl sugars). Further oxidation of glycation items by ROS prompts the arrangement of AGEs, which harms organic tissues by a few instruments (Figure 5).

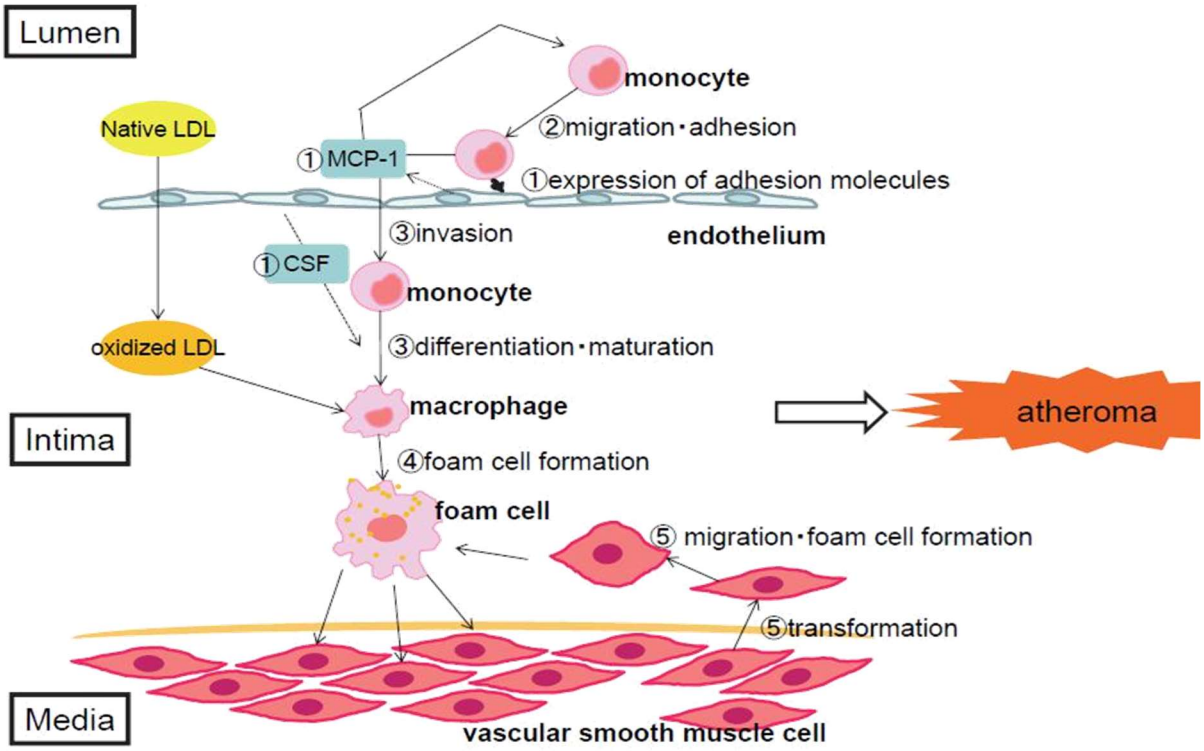

Figure 5: Formation of atherosclerosis in diabetes

Source: Adapted from Katakami (2018)

Plasma protein alteration by AGE precursors is one potential method. As illustrated in Figure 5, modified plasma proteins can bind to AGE receptors found in a variety of cells, including endothelial cells and macrophages, resulting in receptor-mediated generation of oxygen free radicals (Yan, Stern, and Schmidt, 1997), which leads to the development of atherosclerosis.

2.9 Antioxidants

Antioxidants are molecules that can prevent or slow cell damage caused by free radicals, unstable molecules produced by the body in response to environmental and other stresses. Because of ROS' ability to directly oxidize and damage DNA,

proteins, and lipids, oxidative stress has been implicated as a key factor in the onset and progression of late-diabetic complications (Rösen, Nawroth, King, Möller, Tritschler, et al., 2001). Endogenous or exogenous antioxidants that act as free radical scavengers help to maintain steady-state levels of free radicals generated under physiological conditions.

The endogenous cell reinforcements make out of the enzymatic cancer prevention agents like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and non-enzymatic cell reinforcements like diminished glutathione (GSH), α lipoic corrosive, nutrients C and E (Aloh and Ozougwu, 2010; Fukai and Ushio-Fukai, 2011; Lubos, Loscalzo and Handy, 2011).

2.10 Organ studied

2.10.1 Liver

The liver is the glandular organ of vertebrates and some other animals. It comprises of two lobes, right and left hepatic ducts found below the diaphragm.

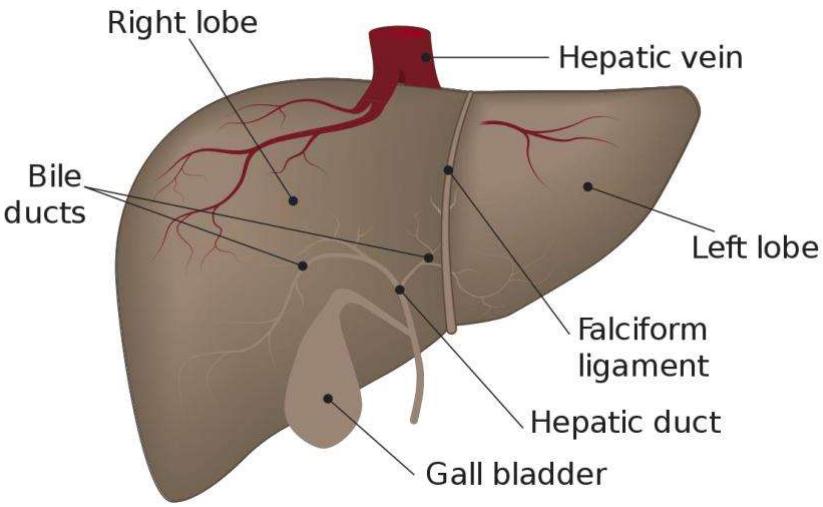

Figure 6: The liver

The liver is significant for endurance and it is basically impossible to make up for the shortfall of liver. It has a wide scope of capacities including detoxification, protein union and creation of biochemical essential for assimilation. It is the primary organ for using, storing, and producing glucose and lipids. Glycogen formation, glucose production from non-sugar carbon sources, and intracellular energy delivery via glycolysis are all part of liver glucose metabolism (Klover and Mooney, 2004).

The key processes in lipid metabolism include fatty acid metabolism, de novo fatty acid synthesis, cholesterol and bile acid production, and lipoprotein assembly. To maintain glucose and lipid homeostatis, these pathways are well-coordinated and controlled in the liver (Raddatz and Ramadori, 2007). Therefore, the liver is the primary site for the anabolic chemical, insulin and its catabolic partner, glucagon. Insulin is delivered from the pancreatic β-cells because of expanded blood glucose focuses. Impedance in insulin affectability and activity in the liver contributes essentially to the pathogenesis of diabetes mellitus (Fritsche, Weigert, Haring, and Lehmann, 2008).

2.12 Biochemical parameters

2.12.1 Serum

Serum is the unmistakable fluid that isolates out from the thickened blood. It contrasts from plasma, the fluid part of ordinary, uncoagulated blood containing platelets, red and white platelets. At the point when the blood is left without anticoagulants like heparin, EDTA and so forth, it clumps. The liquid expressed is named serum since all its fibrinogen and the vast majority of other thickening variables have been taken out. It contains all protein not being used in blood coagulation and all electrolytes, antibodies, chemicals and every single exogenic

substance (Elsohaby, Hou, McClure, Riley, Shaw, 2015). Estimation of serum fixation is helpful in a few fields including clinical examinations.

2.12.2 Malondialdehyde

Malondialdehyde (MDA) is a highly toxic by-product that is used to assess oxidative stress in tissues. It is produced as a by-product of prostaglandin and thromboxane production as well as lipid oxidation. Its plasma concentration rises in people with diabetes, and it's detected in the atherosclerotic plaque deposits that diabetes promotes (Slatter, Bolton, Bailey, 2000). MDA is taken out from the blood by aldehyde dehydrogenase and thio-kinase in the liver, giving it a half-existence of around 2 hours in rodents. MDA's harmfulness comes from its solid reactivity, particularly with proteins and DNA.

2.12.3 Lipid profile

Some studies have detailed that diabetes is related with expansions in serum lipids like complete cholesterol and triacylglycerol, which is identified with critical changes in lipid digestion and design in the sick state (Ayepola, Brooks and Oguntibeju, 2014a; Islam, 2011a). There are three significant kinds of lipoproteins associated with moving cholesterol: high thickness lipoprotein, low thickness lipoprotein and extremely low-thickness lipoprotein (Islam, 2011a). High total cholesterol (TC), high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), and elevated levels of tiny dense LDL particles are common lipid abnormalities in diabetic individuals, referred to as "diabetic dyslipidaemia."

The levels of low density lipoprotein cholesterol (LDL-C) may be slightly elevated or normal. Low-density lipoprotein (LDL-C) helps in the transportation of

cholesterol and fatty oils inside the circulatory system and it is named the 'terrible cholesterol'. Lipid irregularities are normal in people with type-2 diabetes mellitus (Unalacak, Kara, Baltaci, Ozgur, Bucaktepe, 2011) however the example of the various lipids might change between ethnic gatherings, monetary levels, and admittance to medical services (Zimmet, Alberti, and Shaw, 2005).

Moreover, considers in people with type-2 diabetes mellitus have tracked down an expanded relationship between coronary supply route infection (CAD) and high TG and low HDL-C joined, contrasted with the two lipid boundaries evaluated independently (Folli, Corradi, Fanti, Davalli, Paez, et al., 2011). High thickness lipoprotein-cholesterol involved 13% fatty substances, 46% phospholipids, 29% cholesterol esters, 6% cholesterol and 6% free unsaturated fats (Feingold and Grunfeld, 2018). It's the littlest lipoproteins with a thickness of 1.063 – 1.210 mmol/L. It is known as 'great cholesterol' since it eliminates cholesterol from the body cells to the liver for discharge (Eren, Yilmaz, and Aydin, 2012).

2.13 Avocado (*Persea americana*)

Avocado is an enormous tropical normal item with high proportions of bioactive parts, and on account of its clinical benefits, the usage of avocado is extending all throughout the whole world (Araújo, Rodriguez-Jasso, Ruiz, Pintado, and Aguilar, 2018; Migliore, Farina, Dara Guccione, Schifani, 2018). The avocado has distinctive use types, just as business uses in various things like guacamole, chips, frozen yogurt, frozen things, avocado paste, avocado oil and corrective items (Colombo and Papetti, 2019; Palma, Lloret, Puen, Tobar, and Contreras, 2016; Saavedra, Córdova, Navarro, Díaz-Calderón, Fuentealba, et al., 2017). This is basically related with the bioactive mixtures of avocado including unsaturated fats,

dietary fiber, nutrient C, B and E, lutein, colors (carotenoids, chlorophylls, and anthocyanins), and phenolic intensifies This is primarily related with the bioactive combinations of avocado mixtures including dietary fiber, supplement C, B and E, unsaturated fats, lutein, conceals (carotenoids, chlorophylls, and anthocyanins), and phenolic compounds (Lu, Rodríguez, Malgerud, González-Pérez, Martín-Pérez, *et al*., 2015; Saavedra, Córdova, Navarro, Díaz-Calderón, Fuentealba, *et al*., 2017; Wang, Bostic, and Gu, 2010).

Plate 1: Avocado peel

Avocado peel contains carbohydrates (62-73.3%), proteins (4-8.3%), lipids (4.4-9.1%) and fibres (almost 50%) and also it has a great number of bioactive compounds (Colombo and Papetti, 2019). The peel is reported to contain high phenolic content and antioxidant activity. Besides this, the peel has been demonstrated to show effective antimicrobial, antibiotic and anti-inflammatory properties (Adikaram, Ewing, Karunaratne, Wijeratne, 1992; Morais, Rotta, Sargi, Schmidt Bonafe et al., 2015).

In the light of the above mentioned potentials, it is therefore safe to say that it is a promising material for the production of functional foods and pharmaceutical products, and also it can be used as a bio-source for the production of environment

friendly adsorbents (Antasionasti, Riyanto, Rohman, 2017; Kosińska, Karamać, Estrella, Hernández, Bartolomé et al., 2012; Palma, Lloret, Puen, Tobar, Contreras, et al., 2016).

2.13.1 Botany, Agriculture, and Distribution

Avocado has a spot with the gathering of Lauraceae; a dicotyledonous evergreen plant, neighbourhood to Mexico, however really made and ate up around the world (Álvarez, Quezada, Arbelo, et al., 2015; Hurtado-Fernández, Fernández-Gutiérrez, Carrasco-Pancorbo, 2018; Melgar, Dias, Ciric, Sokovic, Garcia-Castello, et al., 2018). The word avocado is used by the Aztecs and got from ahuacatl, while it is called by various names (aguacate, cupandra, avocatier, cura, abacate and palta) in different countries (Araújo, Rodriguez-Jasso, Ruiz, Pintado, Aguilar, 2018; Yahia and Woolf, 2011). It is generally called croc pear, margarine pear and vegetable pear (Hurtado-Fernández, Fernández-Gutiérrez, Carrasco-Pancorbo, 2018).

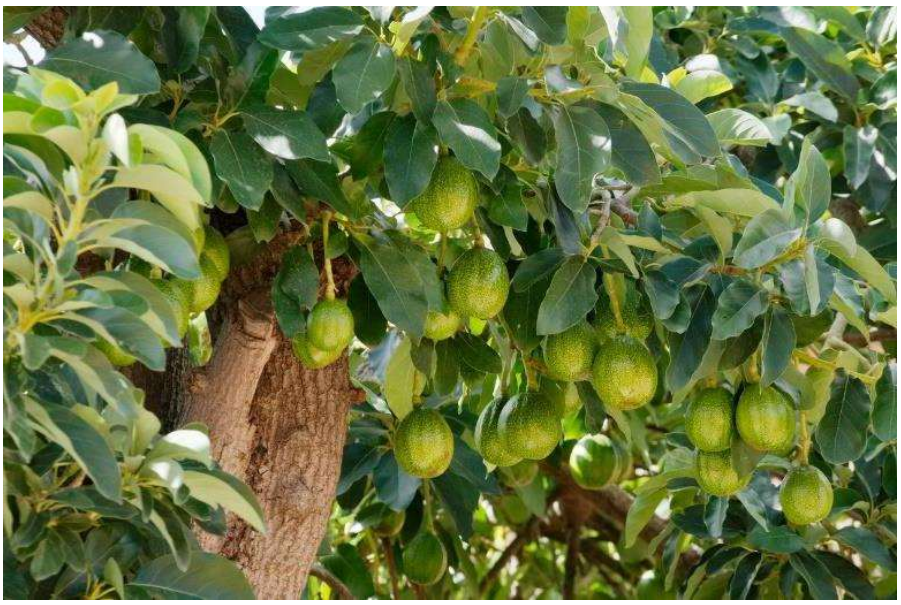

Plate 2: Avocado tree

Botanically, avocado is isolated into three gatherings, with various terms as Mexican (Persea History of the U.S var. drymifolia), Guatemalan (Persea nubigena var. guatemalensis), and West Indian (Persea Yankee folklore var. Yankee folklore). The names rely upon the beginning stages, the normal creating conditions and the properties of the natural item (Araújo, Rodriguez-Jasso, Ruiz, Pintado, Aguilar, 2018; Yahia and Woolf, 2011). There are incalculable avocado combinations with the essential cultivars, for instance, Hass, Fuerte, Bacon, Reed, Gwen, Edranol, Ettinger, Pinkerton, Shepard, Zutano, etc, among them, Hass and Fuerte assortments are the most well-known assortments (Hurtado-Fernández, Fernández-Gutiérrez, Carrasco-Pancorbo, 2018; Mardigan, Santos, dos., Silva, Visentainer et al., 2018; Yahia and Woolf, 2011).

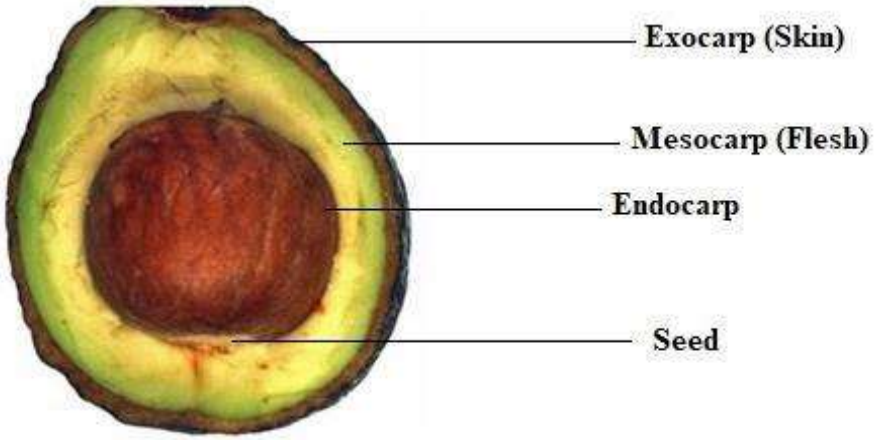

Figure 7: Labelled Avocado Fruit

Avocado is a berry with a large seed in the center and a pericarp that encompasses the skin (exocarp), the edible part (mesocarp), and the seed concealing layer (endocarp) with weights ranging from 120 g to 2.5 kg, rough or smooth surface, thick or thin skin, and an oval to round shape (Araújo, Rodriguez-

Jasso, Ruiz, Pintado, Aguilar, 2018; Hurtado-Fernández, Fernández-Gutiérrez, Carrasco-Pancorbo, 2018). Avocados have a unique ripening characteristic in that they do not ripen on the tree; instead, the ripening process begins after harvesting and takes 5 to 7 days at room temperature.

2.13.3 Phytochemicals of Avocado Peel

Avocado strip is a rich wellspring of phytochemicals in regards to their complete phenolic content territory from 0.6 to 6.8 mg Gallic corrosive same/g test (mg GAE/g test) for the new avocado strip, and between the timespan 120.3 mg GAE/g for the dry avocado strip with the between varietal adjustments. The strip, represents around 18% of the absolute new weight, which contains higher phenolic content and cancer prevention agent movement than the detailed qualities in the eatable part (mash) (Melgar, Dias, Ciric, Sokovic, Garcia-Castello, et al., 2018; Rotta, de Morais, Biondo, dos Santos, Matsushita, et al., 2016; Tremocoldi, Rosalen, Franchin, Massarioli, Denny, et al., 2018; Wang, Bostic, Gu, et al., 2010).

2.13.4 Industries Uses of Avocado

Avocado processing produces a large volume of peel and results in the loss of phytochemically rich components with significant economic worth (Colombo and Papetti, 2019; Coman, Teleky, Mitrea, Martău, Szabo, et al., 2020; Permal, Chang, Seale, Hamid, Kam, 2020). Avocado peel has long been used as a livestock feed (Figueroa, Borrás-Linares, Lozano-Sánchez, Segura-Carretero, 2018). Nonetheless, because the avocado peel has a greater phenolic content than the pulp and seed, it includes essential chemicals for the culinary, pharmaceutical, and other sectors (Kosińska, Karamać, Estrella, Hernández, Bartolomé et al., 2012; Rotta, de

Morais, Biondo, dos Santos, Matsushita, et al., 2016; Tremocoldi, Rosalen, Franchin, Massarioli). For these reasons, recently, avocado peel has been drawing more attention and being investigating more extensively.

Table 2: Pharmacological uses of *P. americana*

Some Pharmacological Uses of different parts of *P. americana*	**References**
Anti-malaria (Leaves)	Falodun *et al*. (2014)
Anti-ulcer (Leaves)	Falodun *et al*. (2014)
Anti-helicobacter (Seed)	Castillo-juarez *et al*. (2009)
Anti-viral (Seed)	De Almeida *et al*. (1998)
Anti-inflammatory (Leaves)	Adeyemi *et al*. (2002)
Antioxidant (Seed)	Folasade *et al.* (2016)
Anti-cancer (Seed)	Oloyede *et al*. (2013)
Anti-diarrheal (Seed)	Odo *et al*. (2013)
Anti-bacterial (Seed)	Idris *et al*. (2009)

Source: Figueroa et al., 2018

Table 3: Commercial uses of *P. americana*

COMMERCIAL USES	REFERENCES
Guacamole	Colombo and Papetti (2019)
Frozen products	Colombo and Papetti (2019)
Avocado paste	Colombo and Papetti (2019)
Avocado oil	Palma *et al*. (2016)
Cosmetic products	Palma *et al*. (2016)
Chips	Saavedra *et al*. (2017)
Ice-Cream	Saavedra *et al*. (2017)

Source: Colombo and Papetti (2019)

Table 4: Applications of the peels of avocado and its bioactive compounds in the industries.

The type of material	Functions	Usage/Potential usage	Reference
Dried avocado peel	Strong antioxidant activity	Creation of phenolic compounds isolates and concentrates	(Saavedra *et al*., 2017)
Avocado peel extracted with acetone/water	Prevention of various oxidation reactions	Protection of crude porcine patties against various kinds of oxidation responses	(Rodríguez-Carpena *et al*., 2011)
Lyophilised avocado peel	Strong antioxidant activity	Food additive or an element for utilitarian food sources	(Kosińska *et al*., 2012)
Avocado peel extracted with various solvents	Strong antioxidant activity	Food preservative as an antioxidant	(Antasionasti *et al*., 2017)
Avocado peel extracted with various solvents	Strong antioxidant and anti-inflammatory activities	Food supplement and functional Food	(Tremocoldi *et al*., 2018)
Avocado peel	Strong antioxidant and antimicrobial activity	Food additive as antioxidant and antimicrobial	(Ortiz-Viedma *et al*., 2018)
Dried avocado peel	Strong antioxidant activity	Usage in tea production	(Rotta *et al*., 2016)
Avocado peel	Adsorption capacity	Production of avocado carbon Peel	(Devi *et al*., 2008)
Avocado peel	Adsorption capacity	Production of carboneous Material	(Palma *et al*., 2016)
Avocado peel	Strong antioxidant activity	Production of nutraceuticals and colouring agents	(Wang *et al*., 2010)

Source: Ortiz-Viedma *et al*., 2018

Avocado peels were dried and utilized to make a unique functional beverage. The avocado peel tea was identical to mate tea in terms of phenolic components, and there was no discernible difference in storage time (Rotta, de Morais, Biondo, dos Santos, Matsushita, et al., 2016). The creation of natural food preservatives is of significant importance since there has been an increase in customer demand for healthier foods (Mark, Anstrom, Sheng, Piccini, Baloch, et al., 2019). Avocado peel extract has been utilized to prevent meat colour degradation and inhibit lipid and protein oxidation, making the peel a possible natural food grade preservative (Rodríguez-Carpena, Morcuende, Estévez et al., 2011).

2.14 Bioactive compounds Characterization

In diabetic rats, quercetin has been shown to lower blood glucose, minimize lipid peroxidation, and enhance SOD, CAT, and GSH levels (Anjaneyulu and Chopra, 2004). Quercetin (QE), a structurally related flavonoid, is a naturally occurring phenolic compound found in a wide range of plants and natural foods. It has been shown to have anti-inflammatory, anti-oxidative, anti-hypertensive, anticancer, antiviral, neuroprotective, hepatoprotective, and anti-diabetic properties (Zamin, Filippi-Chiela, Dillenburg-Pilla, Horn, Salbego, et al., 2009; Szkudelski and Szkudelska, 2011).

Furthermore, QE has been shown to increase the lifetime of model organisms and minimize cell senescence in healthy cells (Machha, Achike, Mustafa, Mustafa, 2007; Zamin, Filippi-Chiela, Dillenburg-Pilla, Horn, Salbego et al., 2009). Supplementing with QE has been shown to help normalize blood glucose levels, increase liver glycogen content and enzymes, and lower serum cholesterol. In diabetes-induced rats, it has also been found to enhance antioxidant status and

prevent oxidative damage, encouraging the regeneration of pancreatic -cell islets and boosting insulin secretion. (Vessal, Hemmati, and Vasei et al., 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

The fruit *Persea americana* (avocado) was obtained from a local market in Omu-Aran, Kwara State, Nigeria, and was identified and authenticated at Botany Department, University of Ilorin, Kwara State. After which the fruit was separated from the peel and dried under sunlight, grinded and stored until extraction.

3.1.2 Chemicals and Reagents

Alloxan, Folin-Ciocalteau reagent, Bovine serum albumin (BSA), Thiobarbituric acid (TBA), Potassium chloride, Ellman's reagent, and other reagents were appropriately sourced for and are of analytical grade. Assay ELISA kits used were of Randox laboratories Ltd., Antrim UK.

3.1.3 Experimental Animals

A total of 36 male Wistar rats (260 ± 15.24g) were purchased from the animal holding unit of the Department of Biochemistry, Landmark University, Omu-Aran, Kwara State. The animals were then housed in cages (12-hour light/dark cycle) with free access to standard rat chow and tap water before the commencement of the study. Ethical approval was also obtained from the Landmark University animal ethical committee Biochemistry department.

3.2 Methods

3.2.1 Polyphenol Extraction

The procedure used for extraction was done in accordance with Sabrina et al. (2019). Microwave assisted extraction was the method used for extracting the polyphenol from the peels. 20g of the powdered avocado peel was measured and placed in a 1000 mL beaker and 500mL of ethanol poured and mixed with the powdered peel. The microwave would have gone through pre-heating for at least 1 min 30 sec. at 60^{o}C, then the mixture in the beaker placed in the microwave and heated for about 20 Mins, left to cool and then filter with Whatman filter paper, the filtrate obtained is then further concentrated using hot water bath to get the polyphenolic peel extract.

3.2.2 Polyphenol Determination

The procedure described by Hagerman, 2012) was adopted in determining the total phenolic content in the aqueous leaf extract of *P. americana*.

Principle:

The oxide group present in the phenol components reacts with phosphormolybdic acid in Folin-Ciocalteau reagent in an alkaline media that produces a blue-colored molybdenum complex. The blue colored complex was measured spectrometrically at 510nm.

Procedure:

0.5 g of the extract (about 0.5 g) of *P. americana* polyphenolic peel was placed in a 50 ml beaker, followed by 20 ml acetone and homogenization for 1 hour. In a 100

ml volumetric flask, the mixture was filtered through Whatman No. 1 filter paper, afterwards 5 mL of acetone was added, followed by the addition of distilled water to make up the marked level. From the resulting solution, 1 ml was pipetted into 50 ml flask and 20 ml of distilled water, 3 ml of phosphomolybdic acid and 5 ml of 23 % Na_2CO_3 were added and mixed. The reaction mixture was left undisturbed for 10 minutes to develop bluish green colour. The absorbance of the sample and standard phenol was read on spectrophotometer at a wavelength of 510 nm. The total phenolic content was extrapolated from a standard curve using gallic acid (0-10 mg/ml) as standard phenol (Figure 20; Avocado polyphenolic peel extractndix II). The amount of total phenolic content was calculated as gallic acid equivalents (GAE, mg gallic acid/g sample) using the gallic acid calibration curve and this formula:

Calculation:

Percentage phenol was calculated using the expression:

$$\%\text{Phenolic} = \frac{\text{Absorbance of Sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of Sample} \times 10{,}000}$$

3.2.3 HPLC Phytochemical screening

The presence of phytocompounds in the peel polyphenolic extracts of P. americana was determined using high-performance liquid chromatography (HPLC). HPLC was performed utilizing a Hitachi equipment and an L-4000 UV detector. According to Lone et al., HPLC analysis was conducted (2015).

Procedure

P. americana ethanolic polyphenolic extracts were filtered using a 0.45 m membrane filter. The ultrasonic technique was used to degas the prepared solvents

and extracts. The phenolic components in *P. americana* peel polyphenolic extract were identified using polyphenol standards. The retention duration and UV absorption spectra of each chemical were compared to those of the standards to identify them.

3.3 Diet formulation

Table 5: Diet formulation and measurement

COMPONENTS	MEASUREMENTS (%)					
Corn flour	60	60	60	60	60	60
Fish meal	15	15	15	15	15	15
Groundnut cake	20	20	20	20	20	20
Vitamin premix	2	2	2	2	2	2
Mineral premix	2	2	2	2	2	2
Fiber	1	1	1	1	1	1
Avocado polyphenolic peel extract	0	0	0	0.0015	0.003	0.006

3.4 Induction of Diabetes

The diabetic rats were fed after a 12-hour fast, after which they were induced using intraperitoneal injection with a single dose of 150 mg/kg Alloxan (Sigma, St. Louis, MO., USA) prepared in 0.1 M of sodium citrate buffer (pH 4.5). After 72 hours of inducing, fasting blood glucose (FBG) level was determined by collecting blood samples from the tip of the tail and to measure the FBG level, a glucometer was used and FBG level 270 mg/dL were considered to be diabetic (Ojo, Ajiboye, Oyinloye, Ojo, Olarewaju, 2014).

3.5 Experimental design

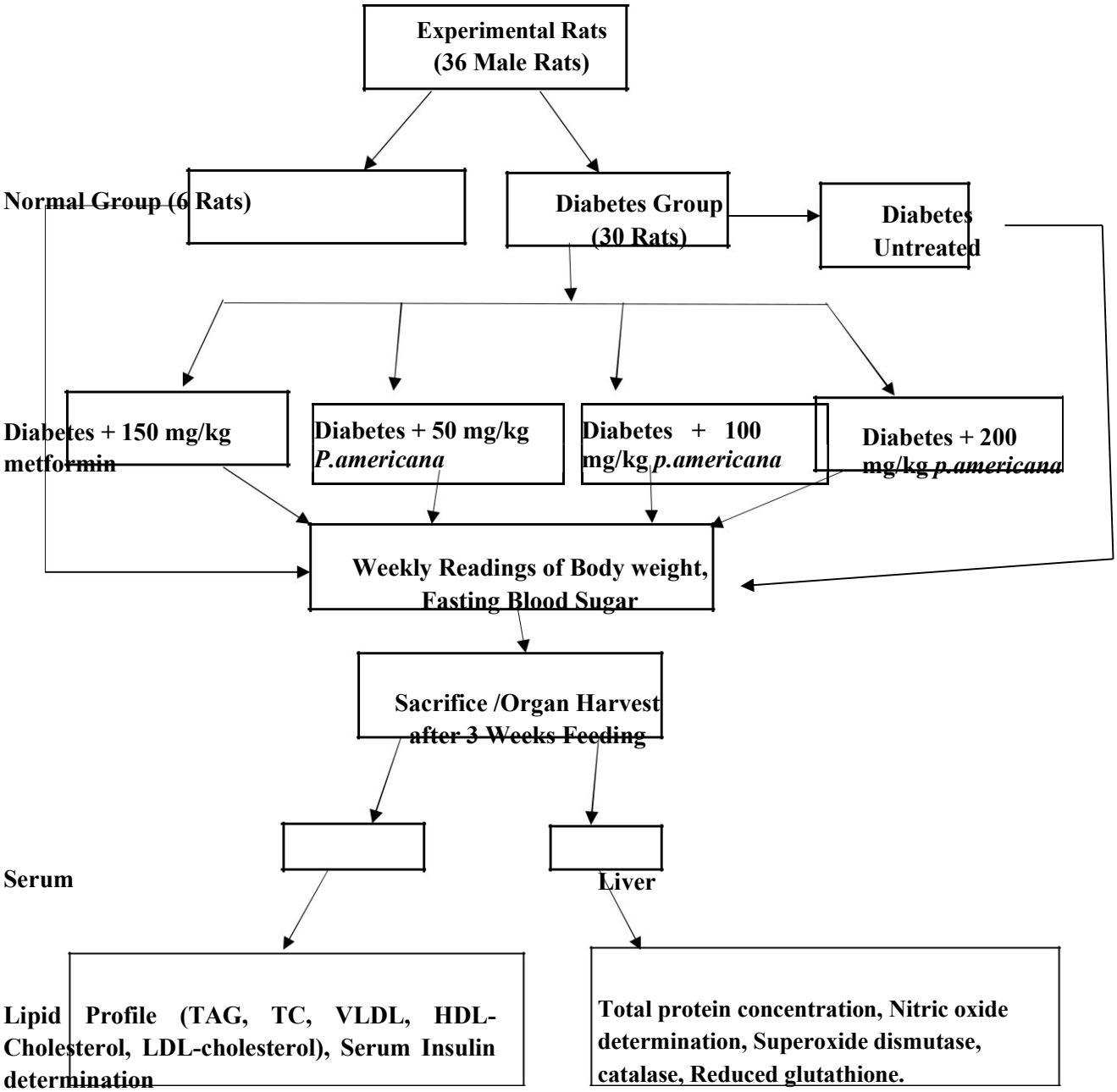

Figure 8: Experimental design of the research work

3.5.1 Animal groupings and feeding

The study employed 36 male Wistar rats, divided into six groups of six rats each. Before being fed, the animals were kept in a temperature and humidity-controlled environment with a 12-hour light-dark cycle and fed a formulated diet. Because the rats were raised in the same environment prior to the induction of diabetes and therapy, they do not need to be acclimatized.

Below is the grouping of the animals:

Group A: Normal rats + distilled water

Group B: Diabetic rat + distilled water

Group C: Diabetic rat + Standard drug metformin (150 mg/kg bw)

Group D: Diabetic rat + fed 50 mg/kg bw of avocado polyphenolic peel extract supplemented diet

Group E: Diabetic rat + fed 100 mg/kg bw of avocado polyphenolic peel extract supplemented diet

Group F: Diabetic rat + fed 200 mg/kg bw of avocado polyphenolic peel extract supplemented diet

3.6 Body weight Determination

The body weight of all animals in each group was determined before the commencement of the treatment, and body weight was measured weekly until the end of the experiment.

3.6 Preparation of serum and tissue supernatants

The blood was collected according to the method described by Ojo, Ajiboye, Oyinloye, Ojo, Olarewaju, (2014). The rats were placed in the desiccator which contains diethyl ether, the blood was then drawn from the jugular veins into plain bottles. The blood was then allowed to clot for 5 minutes. The blood was then centrifuged using a bench top centrifuge at 3000 × g for 10 minutes at -40^{o}C after which the serum was separated using pipettes into a dry clean sample bottle, stored in the freezer for further analysis. The liver, was harvested and blotted with tissue before been weighed, and separated into tubes containing formalin for histology studies. The liver was exercised and placed in 0.25 M sucrose solution (1:5 w/v). They were further homogenized using mortar and pestle in an ice-cold environment, and afterwards centrifuged at 5000 × g for 10 minutes; the supernatant separated and transferred into sample bottles and stored in the freezer until analysis.

3.7 Determination of Biochemical Parameters

3.7.1 Fasting blood glucose

The fasting blood glucose level was determined using the procedure described by Ahmad, Mahmood, Gulzar, Akhtar, Saleem (2011).

Principle

The accu-check gluco machine was used to determine the fasting blood glucose and it uses the glucose-dye-oxidoreductase mediator reaction. The enzyme present on the strip, a mutant variant of quinoprotein glucose dehydrogenase from *Acinetobacter calcoacericus,* as well as a recombinant *E. coli* which converts the

blood sample to gluconolactone. This process then produces a harmless electrical current that the meter on the accu-check interprets for a proper glucose test in mMol/L after which it was converted to mg/dL by multiplying by 18 (conversion factor).

Procedure

The rats were made to go through an overnight fast prior to the test. Blood was collected from the tip of their tail by cutting them with a sterilized blade to avoid contamination. The tail is then gently squeezed in order to get a drop of blood which is dropped on the strip inserted into the machine. After some seconds the machine then displays the glucose level which was further recorded. The blood glucose level was taken every 7 days until the end of the experiment. 5 reading was recorded all together before the induction, after 72 hours, 7^{th} day, 14^{th} day, and 21 days respectively.

3.7.2 Serum insulin

Serum insulin was determined using Mercodia rat insulin ELISA kits (Anderson, Dinesen, Jorgonsen, Poulsen, Roder, 1993).

Principle

The Merocodia Insulin ELISA is a two-site enzyme immunoassay utilizing the direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants of the insulin molecule. Insulin present in the sample binds to anti-insulin antibodies bound to the sample well, while the peroxidase-conjugated anti-insulin antibodies also bind to the insulin at the same

time. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of insulin in the sample. The endpoint is read at a wavelength of 450 nm using a micro-plate reader.

Procedure

Briefly, all reagents and samples were prepared at 25 ºC prior to use. The required amount of enzyme conjugates 1X and wash buffer solution was prepared. The samples, insulin control solutions, and calibrators were also prepared as well as sufficient micro-plate wells to accommodate calibrators and samples in duplicates. A recommended plate plan which comprises Cal 0-5: calibrator solutions (standards); insulin control low, insulin control high and sample were prepared. 25 µl each of calibrators was pipetted into appropriate wells and 100µl of enzyme conjugates 1X solution added into each well and incubated on a plate shaker at 2000 × g for 2 hours at 25 ºC. Each well was washed six times with wash buffer 1X solution and reaction volume discarded by inverting the micro plate over a sink. This was repeated five times to avoid prolonged soaking during the process. 200 µl substrate 3, 3, 5, 5'-tetramethylbenzidine (TMB) was the added into each well. This was incubated for 15 minutes at 25 ºC and 50 µl stop solutions were added to each well. The late was placed on a shaker at 280 × g for 5 minutes to ensuring thorough mixing. The absorbance was read at 450 nm within 3 minutes.

3.8 Antioxidants Determination

3.8.1 Catalase activity

The activity of catalase was determined according to the method described by Winterbourn, Cowden, Sutton (1993).

Principle

The decomposition of hydrogen peroxide by catalase to yield water and oxygen molecules. Ultraviolet light absorption of hydrogen peroxide was read at 240 nm.

Procedure

Test tubes were clean and labelled reagent and sample respectively. Liver samples (10 µl) and 10 µl sucrose solutions (blank) were dispensed into the reagent and sample labelled tubes containing 100µl of cold 6 mM H_2O_2 and then mixed properly. After 3 minutes, the reaction mixture was terminated by adding 20 µl of 3 M $HMnO_4$ (0.01M, 140µl) was added and mixed. The absorbance was read at 480 nm within 30-60 seconds.

Calculation

Catalase activity was calculated and expressed as follows:

$$\text{Catalase activity (Units/mg protein)} = \frac{\Delta A_{240}/\text{min} \times 1000}{43.6 \times \text{TPC} \times \text{V}}$$

Where; ΔA_{240}/min = change in absorbance

1000 = Factor introduced to enable enzyme activity to be expressed in mg equivalent

43.6 = H_2O_2 extinction coefficient

TPC= total protein concentration (mg/ml)

V = volume of the reaction mixture (ml)

3.8.2 Superoxide Dismutase activity

The activity of superoxide dismutase was determined according to the method described by Zelko, Manriani, Folz (2002).

Principle

Superoxide anion, a substrate for superoxide dismutase is produced indirectly in the oxidation of epinephrine at alkaline pH (7.4) by the action of oxygen on epinephrine. As super oxide anion builds, the formation of adrenochrome accelerates because superoxide anion also reacts with epinephrine to form adrenochrome.

Procedure

Briefly, 0.2 ml of liver supernatant was added 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3mM adrenaline. The blank contained 2.5 ml buffer, 0.3 ml of epinephrine and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 secs for 150 secs.

Calculation

Superoxide dismutase was calculated and expressed as follows

Increase in absorbance per minute = $\frac{A_3 - A_0}{2.5}$

Where; A_0 = absorbance after 30 secs and

A_3 = absorbance after 150 secs

2.5 = total time

3.8.3 Glutathione peroxidase activity

GPx activity in the sample was determined according to the method described by Jurkovič, Osredkar, Marc (2008).

Principle

Glutathione peroxidase utilizes two molecules of reduced glutathione (GSH) to catalyse the splitting of one molecule of H_2O_2 and other peroxides ($\bar{}OOH$)**.** The reaction proceeds for a specified period (in minutes) and then terminated by the addition of trichloroacetic acid. The residual GSH in the reaction mixture is then quantified by the addition of Ellman's reagent [(5',5'-dithiobis- (2-nitrobenzoic acid), DTNB]. The decrease in NADPH absorbance read at 340 nm during the oxidation of NADPH to $NADP^+$ is suggestive of GPx activity.

Procedure

The reaction mixture containing 890 µl of 50 mM tris-HCl buffer at pH 8 was mixed with 50 µl of 5 mM NADPH reagents which was constituted in the test tube labelled both blank and sample. Liver samples (50 µl) and 10 µl of 30 mM tertiary

butoxide were added. The change in absorbance of the reaction mixture was read at 340 nm for 3 minutes.

Calculation:

Glutathione peroxidase activity was calculated and expressed as follows:

$$\text{GPx activity (U/mg protein)} = \frac{A_{sample} - A_{blank} \times 1000 \times v \times DF}{6.22 \times V}$$

Where; 6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm

DF = Dilution factor

v = volume of the sample

V = volume of the reaction mixture

1000 = Factor introduced to enable the enzyme activity to be expressed in mg

3.8.4 Reduced Glutathione

The levels of reduced glutathione (GSH) in the samples was determined by the method described by Livingstone and Davis (2007).

Principle

The reduced form of glutathione often comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based on the

development of a relatively stable yellow colour when Ellman's reagent (5′5′-dithiobis –2-benzoic acid) is added to sulfhydryl compound. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was read at 430 nm in a spectrophotometer. Reduced GSH is proportional to the absorbance at 412 nm.

Procedure

Liver sample (0.2 ml) was added to 1.8 ml of distilled water and 3 ml of the precipitating reagent was mixed with the sample. The mixture was then left for 5 minutes and then filtered. Furthermore, 1 ml of the filtrate was added to 4 ml of 0.1M phosphate buffer and finally, 0.5 ml of the Ellmans' reagent was added. A blank was prepared with 4 ml of the 0.1M phosphate buffer, 1 ml of diluted precipitating solution and 0.5 ml of the Ellman's reagent. The absorbance was read at 412 nm. The glutathione (reduced) was read from the calibration curve.

3.8.5 Malondialdehyde

The concentration of MDA was determined by adopting the method described by Reilly and Aust. (2001).

Procedure

Solution of thiobarbituric acid/trichloroacetic acid/hydrochloric acid (TBA/TCA/HCL) was prepared by diluting stock solution in 4-folds. Butylhydroxytoluene was added to the final concentration. The aqueous lipid suspension and blank was combined with the TBA/TCA/HCL reagents in ratio 2:1

(v/v). These were mixed carefully and placed in a boiling water bath for 5 minutes and allowed to cool. It was further centrifuged at 1000 × g for 10 minutes at 37 ºC. Absorbance was read at 535 nm.

Calculation

MDA levels was calculated and expressed as

$$\text{MDA (nmd/ml)} = \frac{A_{sample} - A_{blank} \times DF}{1.56 \times 10_6 \times \text{protein concentration}}$$

Where; A_{sample} = absorbance of the sample

A_{blank} = absorbance of the blank

DF = Dilution factor

3.9. Determination of Lipid profile

3.9.1 Total cholesterol

Total cholesterol was determined by employing the method described by Friedewald, Levy, Fredrickson (1972).

Principle

Cholesterol assay is based on cholesterol esterase hydrolysis hydrolysis of cholesterol esters to form free cholesterol dehydrogenase catalyzed conversion of cholesterol to cholest-4-ene-one, in which NAD is reduced to NADH. The optical density of the formed cholesterol concentration in the sample was then taken.

Cholesterol ester + H_2O ⟶ Cholesterol + fatty acids

(v/v). These were mixed carefully and placed in a boiling water bath for 5 minutes and allowed to cool. It was further centrifuged at 1000 × g for 10 minutes at 37 ºC. Absorbance was read at 535 nm.

Calculation

MDA levels was calculated and expressed as

$$\text{MDA (nmd/ml)} = \frac{A_{sample} - A_{blank} \times DF}{1.56 \times 10^6 \times \text{protein concentration}}$$

Where; A_{sample} = absorbance of the sample

A_{blank} = absorbance of the blank

DF = Dilution factor

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Cholesterol ester + H_2O $\longrightarrow$ Cholesterol + fatty acids

Cholesterol + H_2O + O_2 ⟶ Cholesteroma + H_2O_2

H_2O_2 + 4–Amino antipyrine + P-Hydrobenzoic acid

Coloured Quinonic Derivatives + $4H_2O$

Procedure

Reagent blank, samples and standard was pre-incubated at 37 ºC for 5 minutes. Reagent blank (1000 µL) and samples (10 µL) or standard (10 µL) was pipetted into cuvette and mixed thoroughly. The cuvettes were inserted into the cell holder and stopwatch was started. The absorbance of sample, standard and the reagent blank was read at 500 nm within 60 minutes. The absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank was calculated.

Calculation

Total cholesterol concentration was calculated and expressed as follows:

$$\text{Total Cholesterol (mg/dl)} = \frac{\Delta A \text{ sample} \times \text{Concentration of standard (mg/dl)}}{\Delta A \text{ standard}}$$

Where,

ΔA sample = change in absorbance of sample

ΔA standard = change in absorbance of standard

Concentration of standard = 200 mg/dl

3.9.2 Determination of Triglycerides

Serum Triacylglycerol was determined by adopting the method described by Fossati and Prencipe (1982).

Principle

Lipase produces free fatty acids and glycerol by hydrolyzing serum triglycerides. In the process mediated by glycerol kinase, the glycerol released is phosphorylated by adenosine triphosphate (ATP), producing glycerol-1-phosphate (G-1-P) and adenosine-5'-diphosphate (ADP). Glycerol phosphate oxidase then converts G-1-P to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). The peroxidase-catalyzed reaction of 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (ESPA) with H_2O_2 results in a quinoeimine dye, which is red in color. The content of glycerol (and triglycerides) in the sample is proportional to the rise in absorbance at 540 nm. The reaction is represented by the following equations.

Triacylglycerol + H_2O → glycerol + fatty acids

Glycerol + ATP → glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 → Dihydroxyacetone + phosphate + H_2O_2

4-aminophenazone + 4-chlorophenol → Quinoneimine + HCl + $4H_2O$

Procedure

Test tubes labelled reagent blank, standard and sample were set. 1000µl of working reagent was added to 10 µl of sample. The constituents were mixed properly and

incubated for 10 minutes at 25ºC. The absorbance of the sample and standard was read at 546nm against the blank within 60 minutes.

Calculation

Triglycerides concentration was calculated and expressed as follows;

$$\text{Triglycerides concentration (mg/dl)} = \frac{\Delta A \text{ sample} \times \text{Conc. of standard (mg/dl)}}{\Delta A \text{ standard}}$$

Where,

ΔA sample = change in absorbance of sample

ΔA standard = change in absorbance of standard

Concentration of standard = 200 mg/dl

3.9.3 High density lipoprotein cholesterol

High-density lipoprotein (HDL-C) was determined by adopting the method described by Jacob, Mebane, Bangdiwala, Criqui, Tyroler (1990).

Principle

Low density lipoprotein (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fractions remaining in the supernatant was determined.

Procedure

The procedure involves two steps:

Precipitation Step: Micropipette was used to dispense 500 µl of sample into corresponding test tubes. 1000 µl of the precipitate was also pipetted into all the test tubes via a micropipette, mixed properly and allow to stand for 10 minutes at 25ºC. The mixture was the centrifuged at 1300 × g for 10 minutes. After centrifugation, the clear supernatant was separated and used for the next step.

Cholesterol CHOD-PAP Step: 100 µl of distilled water was dispensed into test tubes labelled reagent blank. This was followed by the addition of 100 µl of the standard into the second test tubes. Later 1000 µl of the reagent was pipetted into the test tubes labelled supernatant. Finally, 1000 µl of the reagent was dispensed into all the different test tubes (reagent, standard and supernatant). They were mixed properly and incubated at 25 ºC for 10 minutes, the absorbance of sample and standard were then read against the reagent at 546 nm.

Calculation

HDL-Cholesterol concentration was calculated and expressed as follows;

$$\text{HDL-C (mg/dl)} = \frac{\Delta A \text{ sample} \times \text{Concentration of standard (mg/dl)}}{\Delta A \text{ standard}}$$

$$\text{LDL-Cholesterol (ng/dl)} = \frac{\text{Total cholesterol} - (\text{Triglyceride}) - \text{HDL-Chol}}{5}$$

$$\text{VLDL-cholesterol} = \frac{\text{Triglyceride}}{5}$$

Where, TG/5 is equivalent to the concentration of VLDL-cholesterol.

ΔA sample = change in absorbance of sample

ΔA standard = change in absorbace of standard

Concentration of standard = 200 mg/dl

3.9.4 Atherogenic index

Atherogenic index (AI) was calculated using the expression described by Liu, Lin, Li, (1999)

$$\text{Atherogenic index} = \frac{\text{Total cholesterol} - \text{High density lipoprotein-cholesterol}}{\text{High density lipoprotein-cholesterol}}$$

3.9.5 Coronary artery index

Coronary artery index was calculated using the expression described by (Boers, Nurmohamed, Doelman, Lard, Verhoeven et al., 2003).

$$\text{Coronary artery index} = \frac{\text{Low density lipoprotein-cholesterol}}{\text{High density lipoprotein-cholesterol}}$$

LDL-cholesterol = [TC – HDL-cholesterol- (TG/5)]

Where TC- total cholesterol, TG- triglyceride, HDL-C – HDL Cholesterol (Friedewald, Levy, Fredrickson 1972).

3.10 Statistical Analysis

Data was expressed as mean ± SEM (standard error of mean). One-way analysis of variance was used to test for significant group differences, statistical analysis of control and experimental groups were performed by Student's t test. GraphPad ™ PRISM8 software package was used for graphical representations.

CHAPTER FOUR

4.0 Results

4.1 Identification of Bioactive principles in avocado polyphenolic peel extract

The HPLC fingerprint of avocado polyphenolic peel extract revealed three peaks corresponding to the presence of p-coumaric acid, quercetin and kaempferol as shown in the chromatogram in and retention time of 19.306, 35.729, and 38.267 min respectively shown in table 6. Quercetin was identified as shown in figure 9 the phytochemical with the highest retention time and spectral characteristics against the standard. The concentrations were determined by calculating the HPLC peak areas, which are proportional to the amount of analyte in a peak and presented as the mean of three determinations which were highly repeatable.

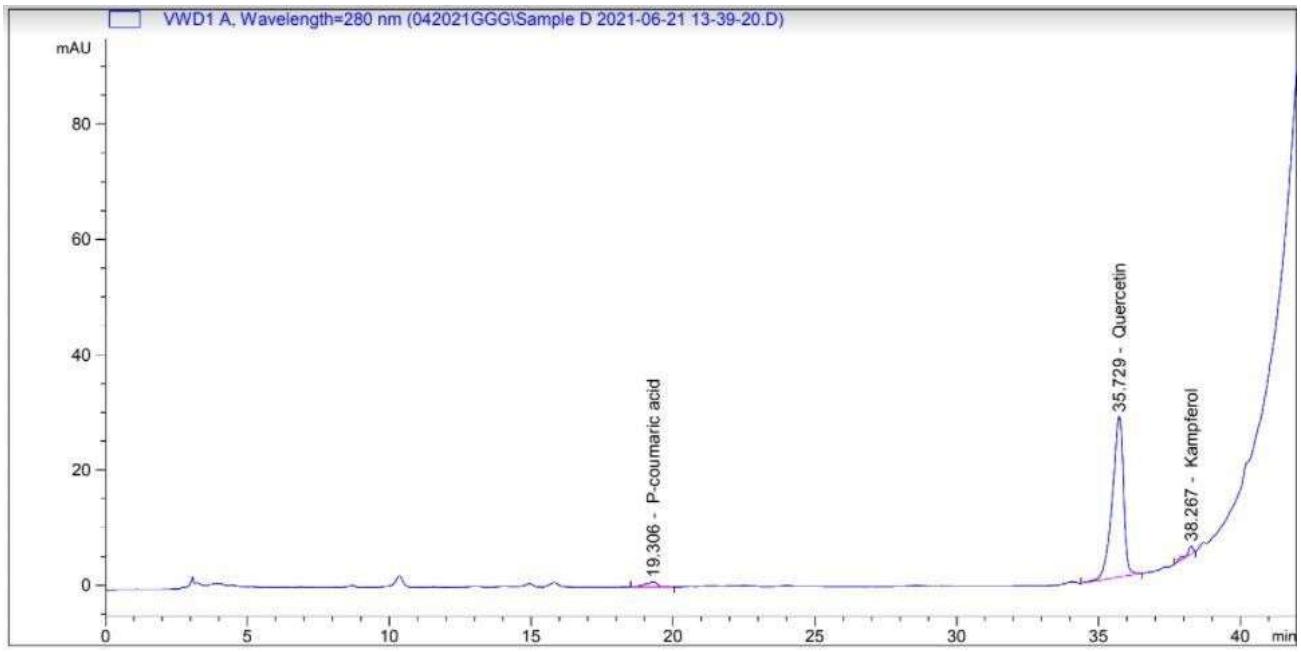

Figure 9: Chromatogram of *P. americana* polyphenolic peel extracts

Table 6: Identification of Bioactive Principles in avocado polyphenolic peel extract

S/N	Compound	Retention time (min)	Amount (ppm)
1	P-coumaric acid	19.306	$8.83002e^{-2}$
2	Quercetin	35.729	41.58143
3	Kampferol	38.267	1.23360

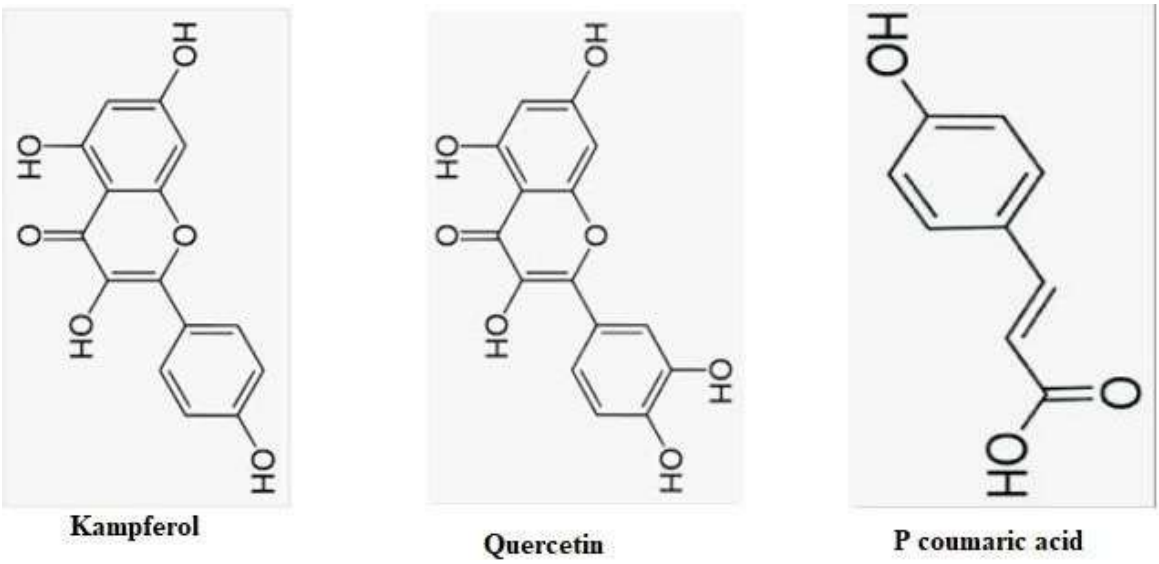

Figure 10: Phytochemical compounds in *P. americana* polyphenolic peel extract

4.2 Body Weight of diabetes male Wistar rats fed avocado polyphenolic peel extract supplemented diet

Table 7 shows the body weight of the animals before treatment, during treatment and after treatment. The diabetic control group and diabetic treated group all experienced a significant decrease in body weight when compared to the normal control group and standard drug group. The diabetic control group shows higher percentage of weight loss at the end of the feeding period (-41.99%) when compared to the normal control group (+15.17%), and standard drug group (+3.23%). The diabetic groups fed with 50 mg/kg bw, 100 mg/kg bw, 200 mg/kg bw avocado polyphenolic peel extract supplemented diet had a positive effect on the body weight of the diabetic rats having a weight loss percentage of about -9.82%, -4.36%, -3.07% when placed in comparism with the diabetic group that has a weight loss of -41.99%.

Table 7: Body weight (g) of diabetic male Wistar rats after feeding with avocado polyphenolic peel extract supplemented diet

GROUPS	BEFORE TREATMENT (g)	AFTER 7 DAYS (g)	AFTER 14 DAYS (g)	AFTER 21 DAYS (g)	% WEIGHT CHANGE
Normal Control	180.74 ± 10.86^{a}	181.82 ± 12.37^{ab}	181.51 ± 13.23^{abc}	208.16 ± 14.76^{abc}	+15.17
Diabetic Control	309.77 ± 20.94^{d}	109.88 ± 35.16^{a}	192.10 ± 37.82^{a}	179.67 ± 36.18^{a}	-41.99
Diabetic + Metformin (150 mg/kg bw)	190.89 ± 7.97^{a}	181.66 ± 50.33^{ab}	173.96 ± 42.46^{a}	197.06 ± 42.62^{a}	+3.23
Diabetic + 50 mg/kg bw avocado polyphenolic peel extract	222.51 ± 13.77^{ab}	217.54 ± 49.74^{ab}	195.10 ± 55.68^{ab}	200.65 ± 58.91^{ab}	-9.82
Diabetic + 100 mg/kg bw avocado polyphenolic peel extract	267.86 ± 14.66^{c}	257.71 ± 59.74^{ab}	248.88 ± 61.13^{ab}	256.19 ± 63.35^{ab}	-4.36
Diabetic + 200 mg/kg bw avocado polyphenolic peel extract	288.27 ± 19.41^{cd}	275.54 ± 60.21^{ab}	260.56 ± 66.54^{abc}	279.41 ± 67.36^{ab}	-3.07

Data are presented as the mean ± SEM of 6 replicates.

[a-d] Values with different letters along a column for a given parameter are significantly ($p < 0.05$).

4.3 Fasting Blood glucose (FBG) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The fasting blood glucose levels of the diabetic rats shown in Table 8 below reveals that the FBG after 72 hours of induction, indicate an increase in the blood glucose level which is a confirmation of the development of diabetes. After feeding for a period of 7 days, the FBG shows a slight decrease in the levels of glucose in their blood when compared to the diabetic control group which remained higher than all other groups. After 14 days, the blood sugar levels were further reduced significantly ($p < 0.05$) respectively in all the diabetic groups fed with avocado polyphenolic peel extract supplemented diet compared to the diabetic control group. And after 21 days, all groups fed with avocado polyphenolic peel extract supplemented diet experienced a normal FBG levels but the group treated with the standard drug (metformin) experienced the lowest when compared to the diabetic control group. Among all diabetic groups fed with avocado polyphenolic peel extract supplemented diet, the group fed with 100 mg/kg experienced the lowest level of FBG compared to the groups treated with 50 mg/kg and 200mg/kg respectively.

Table 8: Fasting blood glucose concentration (mg/dL) of Diabetic Male wistar rat after feeding with avocado polyphenolic peel extract supplemented diet

Groups	Before induction (mg/dL)	After 72 hours (mg/dL)	After 7 days (mg/dL)	After 14 days (mg/dL)	After 21 days (mg/dL)
Diabetic Control	96.00 ± 5.13[a]	381.10 ± 54.38[a]	381.10 ± 54.38[a]	490.28 ± 11.76[b]	407.92 ± 24.56[b]
Diabetic + Metformin (150 mg/kg bw)	87.60 ± 1.59[a]	327.66 ± 16.73[a]	305.12 ± 16.73[ab]	210.06 ± 55.43[a]	156.00 ± 30.85[a]
Diabetic + 50 mg/kg bw avocado polyphenolic peel extract	94.11 ± 3.16[a]	350.40 ± 35.35[a]	325.40 ± 35.35[ab]	240.42 ± 10.81[a]	193.10 ± 36.62[a]
Diabetic + 100 mg/kg bw avocado polyphenolic peel extract	89.40 ± 3.00[a]	345.06 ± 3.31[a]	325.06 ± 3.31[ab]	208.44 ± 18.49[ab]	160.80 ± 21.36[a]
Diabetic + 200 mg/kg bw avocado polyphenolic peel extract	100.85 ± 7.30[a]	325.80 ± 40.57[a]	309.15 ± 40.57[ab]	232.80 ± 67.81[a]	184.97 ± 42.42[a]

Data are presented as the mean ± SEM of 6 replicates.

[a-b] Values with different letters along a column for a given parameter are significantly ($p < 0.05$).

4.5 Total Protein Concentration of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The liver total protein concentration of the experimental groups is as shown in figure 11. The non-diabetic control group shows a significance difference when compared to the diabetic control group, diabetic 50 mg/kg but no significance difference among all other groups (treated or untreated). The diabetic control group shows a significant difference ($p<0.05$) when compared to the diabetic 100 mg/kg and non-diabetic 100 mg/kg but shows no significant difference in all other groups.

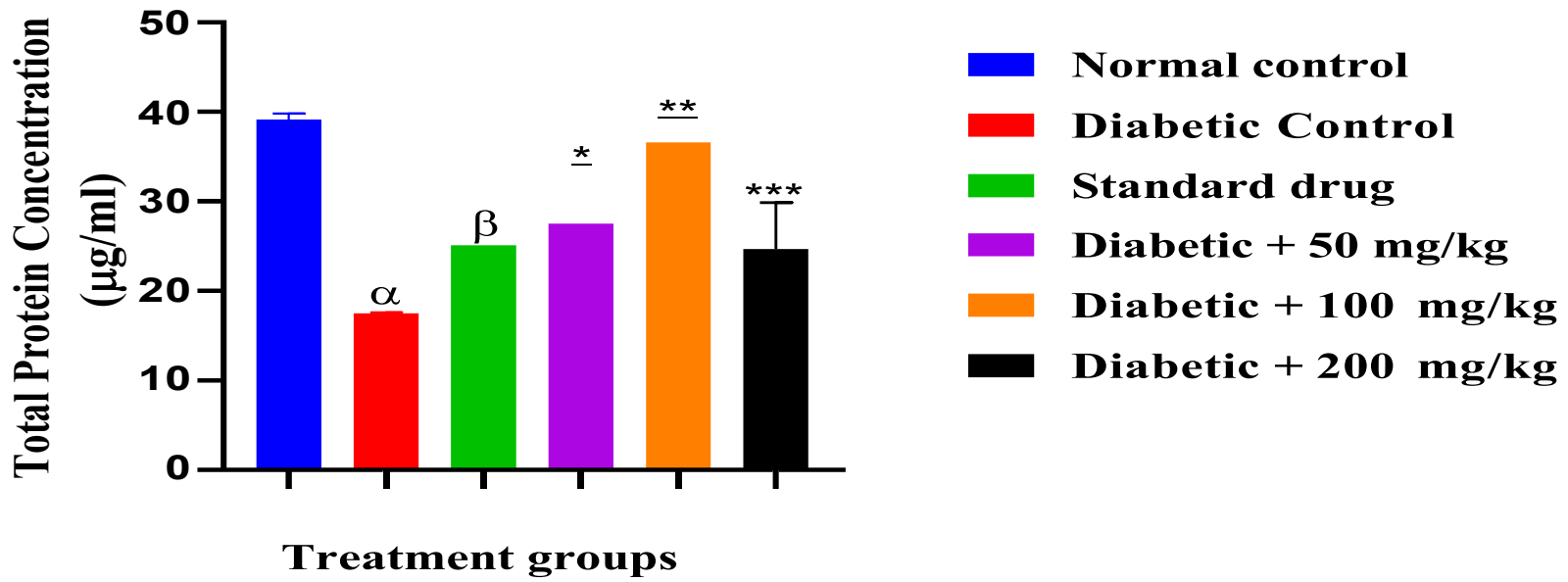

Figure 11: Total protein concentration of experimental animals after feeding avocado polyphenolic peel extract supplemented diet

Data are expressed as mean ± SEM of 6 replicates.

'α' values shows a significant difference to the normal control; 'β' values shows a significant difference to the diabetic control; '*', '**', '***' values shows that there is significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.6 Nitric oxide levels of diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The nitric oxide concentration of the treated and feeding groups in the liver are shown in Table 11, from the result it shows that there is a significant difference ($p<0.05$) between the normal control and diabetic control. A significance ($p<0.05$) difference was also observed between the diabetic control group and the other groups at different feeding concentration of avocado polyphenolic peel extract. There was no significance difference between the standard drug group and the diabetic group fed with 200 mg/kg bw of avocado polyphenolic peel extract supplemented diet.

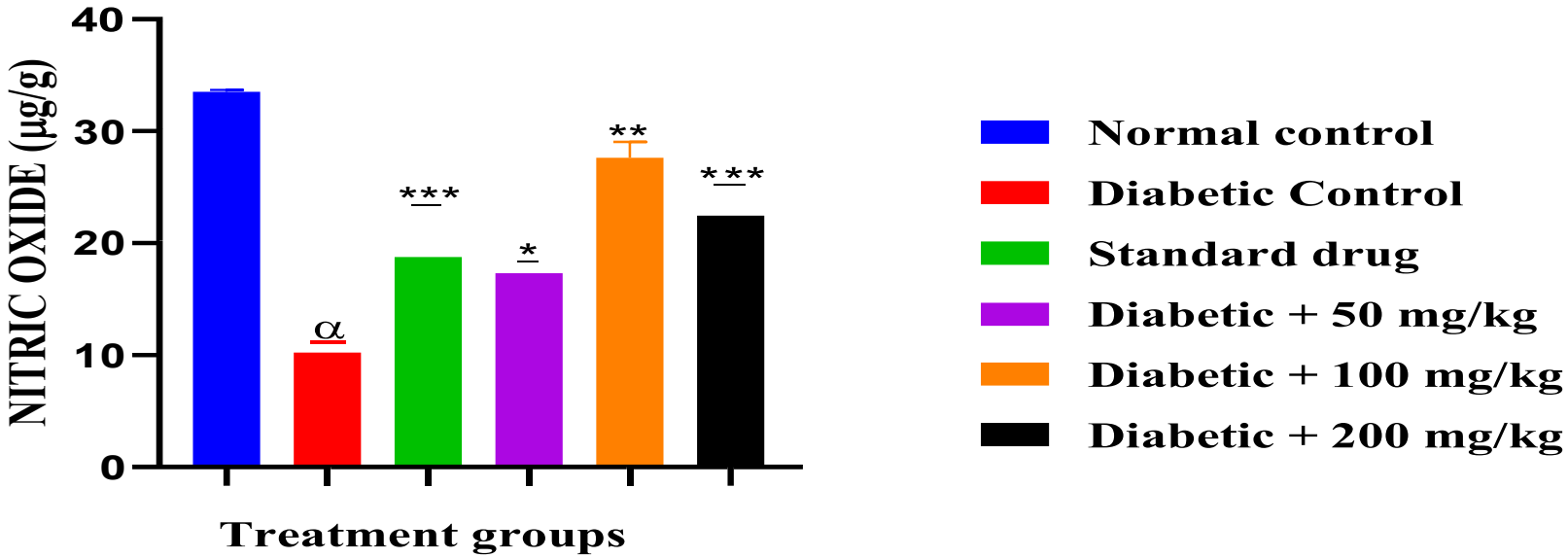

Figure 12: Nitric oxide concentration of experimental animals after feeding with avocado polyphenolic peel extract supplemented diet

Data are expressed as mean ± SEM of 6 replicates.

'α' values shows a significant difference to the normal control; '*', '**', '***' values show significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.7 Reduced glutathione levels of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The liver GSH levels as shown in figure 13 shows that the normal control shows a significant difference from the diabetic control group and the normal rats fed diet supplemented with 50 mg/kg bw of avocado polyphenolic peel extract. The diabetic control group was significantly ($P<0.05$) different compared to the other groups. The standard drug group shows no significant difference across all groups except for the diabetic group treated with 100 mg/kg. From the diabetic treated groups there was significance difference between the 100 mg/kg and 200 mg/kg treated groups respectively.

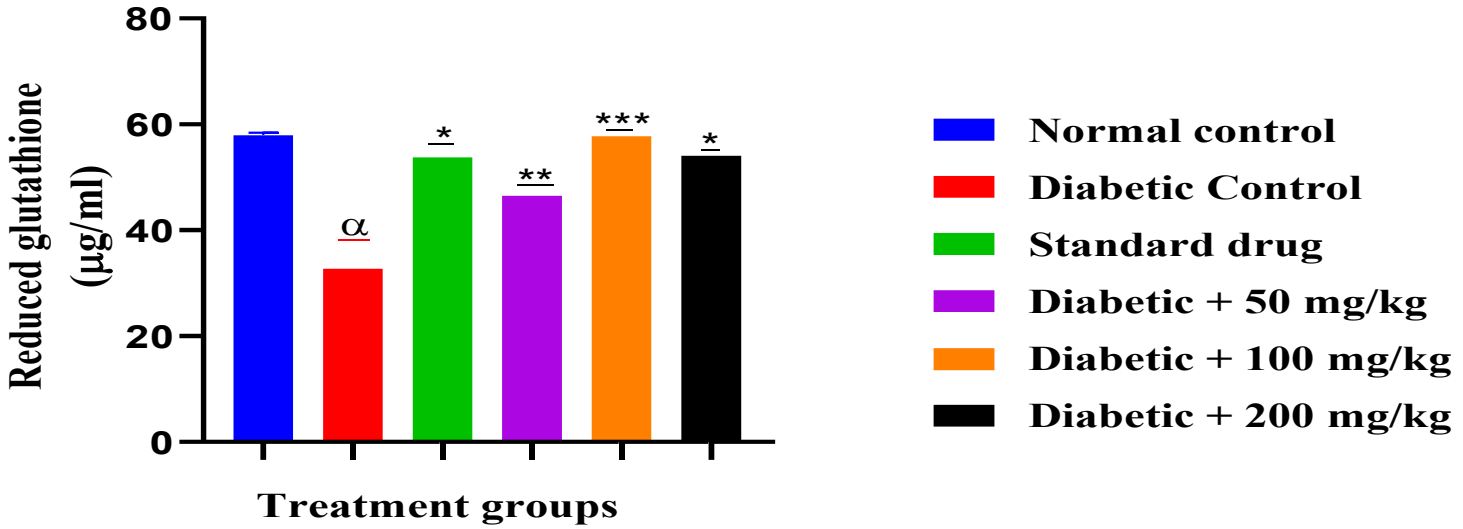

Figure 13: Reduced Glutathione level of experimental animals after feeding with avocado polyphenolic peel extract supplemented diet

Data are expressed as mean ± SEM of 6 replicates.

'α' values shows a significant difference to the normal control; '*', '**', '***' values show significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.8 Lipid Peroxidation (MDA) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The MDA levels in the liver of the various treated and untreated groups is shown in figure 14 below, the normal control indicates a significantly ($p<0.05$)difference when placed in comparism to the diabetic control, the normal 50 and 100 mg/kg respectively but shows no significant ($p<0.05$) difference when compared to the normal 200 mg/kg. The diabetic control is significantly ($p<0.05$) different to the standard drug and the normal 100 mg/kg, but in comparism to the diabetic 50 and 100 mg/kg respectively, there was no significant ($p<0.05$) difference recorded. The standard drug in comparism to the diabetic 50, and 200 mg/kg shows no significant ($p<0.05$) difference but the reverse is the case when compared to the diabetic 100, normal 50, 100, and 200 mg/kg which indicates a significance difference. The diabetic 100 mg/kg shows a significant ($p<0.05$) difference to the diabetic 50 and 100 mg/kg.

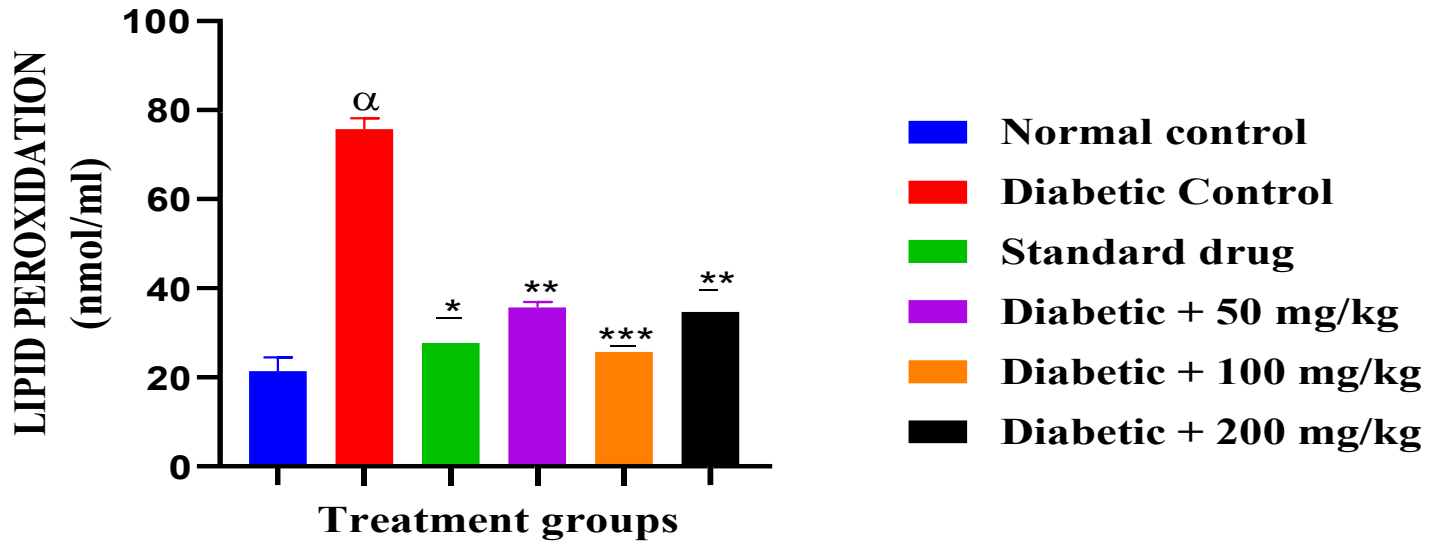

Figure 14: Lipid peroxidation activity of experimental animals after treatment with *P. americana* polyphenol extract supplementation

Data are expressed as mean ± SEM of 6 replicate.

'α' values shows a significant difference to the normal control; '*', '**', '***' values show significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.9 Superoxide dismutase (SOD) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The SOD activity in the liver as shown in figure 15 shows a reduction in the activity significantly ($p<0.05$) in the diabetic control rats when compared to the normal control and other treatment groups. Diabetic rats fed with 100 mg/kg avocado polyphenolic peel extract supplemented diet showed a significantly ($p<0.05$) higher level of SOD activity compared to those treated with the standard drug, as well as 50 and 200 mg/kg avocado polyphenolic peel extract supplemented diet.

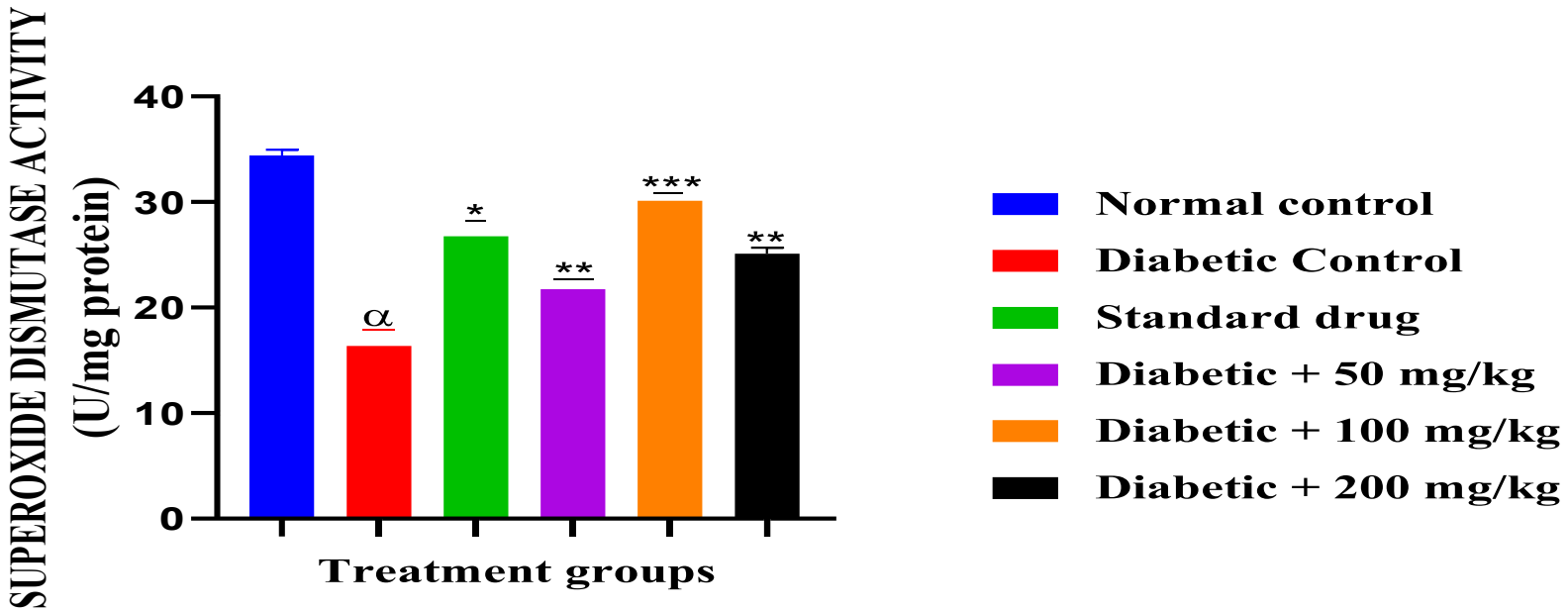

Figure 15: Superoxide dismutase activity of experimental animals after feeding with avocado polyphenolic peel extract supplemented diet

Data are expressed as mean ± SEM of 6 replicates.

'α' values shows a significant difference to the normal control; '*', '**', '***' values show significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.10 Serum catalase Activity of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The serum catalase activity is recorded and shown in figure 16 there was significant ($p<0.05$) reduction in the activity of the diabetic control rat group and diabetic rat groups fed 50mg/kg avocado polyphenolic peel extract supplemented diet compared to the normal control rats. Diabetic rats treated with standard drug and those fed with 200 mg/kg avocado polyphenolic peel extract supplemented diet showed significantly ($p<0.05$) higher CAT activity compared to the diabetic control.

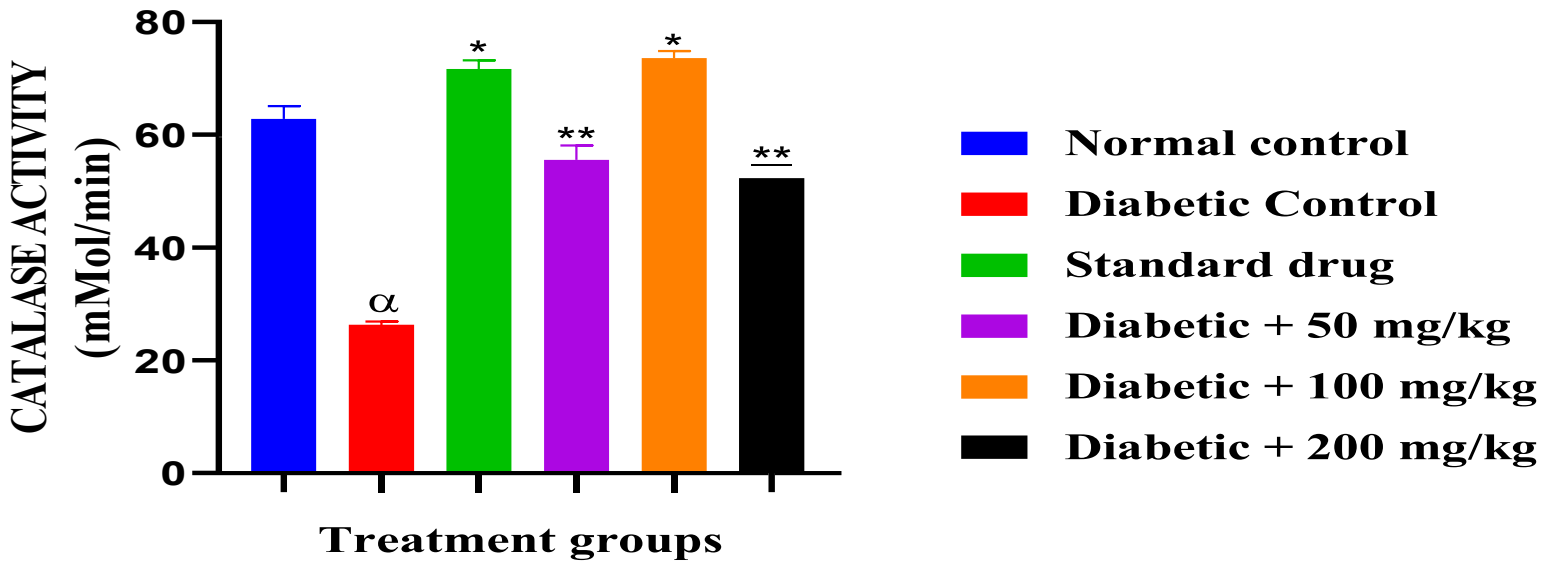

Figure 16: Serum catalase activity of experimental animals after feeding with avocado polyphenolic peel extract supplemented diet

Data are expressed as mean ± SEM of 6 replicates.

'α' shows a significant difference to the normal control; '*', '**', '***' shows that there is significant ($p<0.05$) difference across all diabetic groups fed with avocado polyphenolic peel extract supplemented diet.

4.11 Lipid Profile of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The serum lipid profile is shown in Table 9 below after the induction of diabetes into the diabetic control animals, this resulted to an increase significantly ($p<0.05$), in the levels of total cholesterol, triglycerides, LDL-Cholesterol, VLDL-C, calculated AI and CRI whereas HDL-C was lowered significantly ($p<0.05$) (Table 9). After the treatment with avocado polyphenolic peel extract, was shown that there was significant ($p<0.05$) reduction in the levels of total cholesterol, triglycerides, LDL-Cholesterol, VLDL-C, AI and CRI and elevations in HDL-C content of rat when compared with the non-diabetic distilled water treated control rats. Moreover, the standard drug group treated with metformin significantly ($p < 0.05$) reduced the levels of TC, TG, LDL-Cholesterol, VLDL-C, AI and CRI as well as increased the concentration of HDL-C (Table 9).

Table 9: Serum Lipid Profile Concentrations (mg/dL) of Diabetic Male Wistar rats after feeding with avocado polyphenolic peel extract supplemented diet

Groups	TC	HDL	LDL	VLDL	TAG	A I	C A I
Diabetic Control	14.47 ±1.26^{b}	6.28± 0.56^{a}	7.05±1.86^{b}	0.77 ± 0.05^{a}	3.88±0.26^{a}	1.21±0.38^{b}	1.09 ± 0.53^{b}
Diabetic + Metformin (150 mg/kg bw)	11.57±0.80^{ab}	8.62± 0.09^{b}	2.23±0.69^{a}	0.72 ± 0.02^{a}	3.63±0.08^{a}	0.34±0.08^{a}	0.25 ± 0.11^{a}
Diabetic + 50 mg/kg bw avocado polyphenolic peel extract	10.8 ±0.66^{ab}	7.54±0.05^{ab}	2.68±0.73^{a}	0.65 ± 0.02^{a}	3.26±0.12^{a}	0.44±0.10^{a}	0.34 ± 0.14^{a}
Diabetic + 100 mg/kg bw avocado polyphenolic peel extract	10.26±0.80^{ab}	6.84±0.19^{ab}	2.67±0.63^{a}	0.75 ± 0.02^{a}	3.74±0.09^{a}	0.50±0.08^{a}	0.41 ± 0.11^{a}
Diabetic + 200 mg/kg bw avocado polyphenolic peel extract	10.40±0.75^{ab}	6.74±0.47^{ab}	3.03±0.23^{a}	0.63 ± 0.03^{a}	3.15±0.16^{a}	0.54±0.01^{a}	0.43 ± 0.01^{a}

Data are presented as the mean ± SEM of 6 replicate.

$^{a-b}$ Values with different letters along a column for a given parameter are significantly different at $p < 0.05$. TC, Total cholesterol; TG, Triglyceride; LDL-cholesterol, Low density lipoprotein-cholesterol; HDL-cholesterol; High density lipoprotein-cholesterol, AI; atherogenic index, CAI; coronary artery index.

4.12 Serum, insulin, Homeostasis model assessment-insulin resistance *(*HOMA-IR*),* Homeostasis model assessment- β (HOMA-β) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The serum insulin, HOMA-β levels, and HOMA-IR levels are calculated and shown in Table 9; there was a significant ($p<0.05$) increase in the level of serum insulin as well as the HOMA-IR in the diabetic control group compared to the normal control group and all other groups fed with avocado polyphenolic peel extract supplemented diet. While in the diabetic group fed with avocado polyphenolic peel extract supplemented diet observed a decrease in their serum insulin levels as well as their HOMA-IR levels and are significantly ($p<0.05$) different from each other. HOMA-β levels in the diabetic control shows a significant ($p<0.05$) decrease when compared to the normal control as well as the other groups fed with avocado polyphenolic peel extract supplemented diet.

Table 10: Serum, insulin, Homeostasis model assessment-insulin resistance (HOMA-IR,), Homeostasis model assessment- β (HOMA-β) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

GROUPS	SERUM INSULIN	HOMA-IR	HOMA-β
Normal Control	1.29 ± 0.0004^{a}	0.3 ± 0.0001^{a}	11.22 ± 0.0001^{a}
Diabetic Control	2.05 ± 0.0002^{g}	2.1 ± 0.0004^{b}	2.14 ± 0.0004^{b}
Diabetic + Metformin (150 mg/kg bw)	1.76 ± 0.0004^{e}	0.7 ± 0.0002^{c}	6.81 ± 0.0002^{c}
Diabetic + 50 mg/kg bw avocado polyphenolic peel extract	1.41 ± 0.0002^{b}	0.7 ± 0.0002^{c}	5.19 ± 0.0002^{c}
Diabetic + 100 mg/kg bw avocado polyphenolic peel extract	1.64 ± 0.0004^{d}	0.7 ± 0.0002^{c}	6.04 ± 0.0002^{c}
Diabetic + 200 mg/kg bw avocado polyphenolic peel extract	1.58 ± 0.0004^{h}	0.7 ± 0.0002^{c}	4.66 ± 0.0002^{c}

Data are presented as the mean ± SEM of 6 rats.

[a-h] Values with different letters along a column for a given parameter are significantly different from each other. HOMA-IR, Homeostasis model assessment-insulin resistance; HOMA-β, Homeostasis model assessment- β

4.12 DISCUSSION

Plants have been a vital source of medicine and presently, many plants have been proven to be effective against various diseases. It is well known that either directly or indirectly, drugs are manufactured on the bases of plants. Different kinds of studies have shown that a vast species of plant extracts showed effectiveness in lowering the blood glucose levels in alloxan-induced diabetic animals (Yakubu, Salimon, Abdul-Rasheed, Opakunle, Jimoh, 2014). Diet has also been identified as a major strategy in the management of diseases. Research have stated that *P.americana* peel as a rich source of antioxidant i.e. it possess antioxidant properties. Metformin, a reference drug that is known for its anti-diabetic properties is commonly used in several research studies to compare the accuracy and potency of various acclaimed compounds.

4.12.1 Secondary Metabolites

The anti-diabetic properties of several medicinal plants have been related to bioactive principles like phenols (Oluba, 2019).

4.12.2 Body Weight of diabetes male Wistar rats fed avocado polyphenolic peel extract supplemented diet

Induction of diabetes with alloxan is characterized by the loss of body weight that is caused by muscle wasting, loss of adipose tissue and the breakdown of tissue proteins that occurs in diabetic rats (Adiga, Bairy, Meharban, Punita, 2010). In this research work, loss ofbody weight was observed in the diabetic control group, but after the feeding with avocado polyphenolic peel extract supplemented diet, there was an improvement in their body weight (Table 7) which implies that the

supplementation had a positive effect on the diabetic animals at the end of the treatment period (21 days).

4.12.3 Fasting Blood glucose (FBG) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

Alloxan induction led to the destruction of β-cells in the islet of the langerhans located in the pancreas which in turns result in the rise of the blood glucose levels, which leads to the functional and structural changes in target organs. Because the loss of the β-cells in the pancreas, there is a massive reduction in insulin secretion which led to the rise in the level of glucose in the blood. In this research work, avocado polyphenolic peel extract supplemented diet as shown displays the release of the effect of alloxan on the blood glucose level by increasing the insulin production leading to the reduction in blood glucose level and this would suggests that the supplementation as led to the regeneration/restoration of the β-cells in the pancreas and aiding the release of the newly produced insulin into the blood stream thereby increasing the sensitivity of cell receptors to insulin indicating that the polyphenolic supplementation has anti-diabetic properties (Ajiboye, Mohammed, Bello, Yusuf, Ibitoye et al., 2016).

4.12.4 Antioxidant enzymes of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

Catalase (CAT), Superoxide dismutase (SOD), and Reduced glutathione (GSH) are antioxidant enzymes that protect the biological system from oxidative stress by maintaining physiological oxygen and hydrogen peroxide concentrations. Exposure to alloxan can cause oxidative stress, which these antioxidants can

counteract. They do so by increasing the dismutation of oxygen radical and clearing organic peroxides that was generated due to the exposure (Pari and Letha 2005). SOD is responsible for catalyzing the conversion of highly reactive superoxide radical (O_2^-) to a less toxic hydrogen peroxide and oxygen. CAT and GSH are both responsible for acting on the hydrogen peroxide by scavenging them and removing them from the system. It is obvious that after the exposure to alloxan which induced diabetes, disrupt the activities of hepatic antioxidant enzymes and by so doing resulting in the decrease of the antioxidant activities because of the generation of ROS by alloxan. It could also possibly be a case that the free radicals formed inhibited the activities of these antioxidant enzymes. Due to the inability of the antioxidants to defend the system, it leads to ROS mediated damage. However, the feeding with avocado polyphenolic peel extract supplemented diet significantly reduced the imbalance between the generation of ROS and antioxidant enzyme activities in diabetic rats. This could be as a result of the decreased oxidative stress because the antioxidants scavenge free radicals and maintain normal levels of antioxidant defense system by preventing the reactive oxygen species from causing more damage to membrane lipids (Dumanović, Nepovimova, Natić, Kuča, Jaćević, 2020).

4.12.5 Lipid peroxidation (MDA) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

In biological membranes, lipid peroxidation is considered as one of the major mechanisms of cell injury when it comes to aerobic organisms because they are subjected to oxidative stress. The series of reaction of lipid peroxidation increases the amounts of free radicals in the cell which lead to further peroxidation (Yakoub,

Abdehedi, Jridi, Elfalleh, Nasri et al., 2018). Generation of ROS by metal oxidants such as iron, may be involved in the cell damage which occurs in the pathology of humans. Fe^{2+} could be the initiating factor of lipid peroxidation and suppress the species responsible for the initiation of the peroxidation.

There is therefore a high possibility that the depletion of iron could decrease oxidative stress throughout the entire body. According to figure 14 the increased MDA levels in the presence can catalyze one-electron transfer reactions which generates ROS. The reverse was observed after the feeding with avocado polyphenolic peel extract supplemented diet could be attributed to the phytochemicals present in the extract; that would have formed complexes with the Fe^{2+} and preventing them from catalyzing the initiation of lipid peroxidation.

4.12.6 Lipid Profile of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

One of the major hallmarks of diabetes dyslipidemia is attributed to high serum cholesterol, triglyceride, LDL-C and low level of HDL-C (Moradian and Behnia, 2009). Another important parameter is the high level of LDL-C and atherogenic index which is caused by alloxan induced dyslipidemia and thus, suggest a high-level occurrence of cardiovascular disease (Yakubu, Salimon, Abdul-Rasheed, Opakunle, Jimoh, et al., 2014).

According to Table 9 the high level of cholesterol, triglyceride and LDL-C in diabetes control group could be due to an increase in the mobilization of the free fatty acid depots and this signifies hyperglycemia. It characterized the diabetic state and therefore be regulated as a consequence of uninhibited actions of lipolytic

hormones on the fat depots. Furthermore, the ability of avocado polyphenolic peel extract supplemented diet to reduce the hyperlipidemic condition in all the treated rats, suggests the potential of the diet to reverse the damaged lipid metabolism normally associated with diabetes mellitus. And this could be due to the activation of lipases which are essential for the hydrolysis of lipids (Oyedem, Adewusi, Aiyegoro, Akinpelu, 2011).

4.12.7 Serum, insulin, Homeostasis model assessment-insulin resistance *(*HOMA-IR*),* Homeostasis model assessment- β (HOMA-β) of Diabetic Male Wistar rats fed avocado polyphenolic peel extract supplemented diet

The diabetic treated group experienced a decrease in serum insulin and this could be due to the pancreatic β-cell dysfunction in response to high blood glucose level. However, after feeding with the diet, all treated groups experienced an improvement significantly ($p < 0.05$). This could also be attributed to the regeneration /restoration of the pancreatic β-cell, which was supported by the higher homeostatic assessment score of β-cells (HOMA- β) in the treated groups. The lower HOMA-IR index in the treated group could be attributed to the restorative effect of the supplemented diet which showed an improvement of insulin sensitivity as well as the stimulation of peripheral glucose absorption. This effect could be attributed to the presence of flavonoid, quercetin identified in *P. americana* polyphenolic peel extract since quercetin have been reported to promote the regeneration of pancreatic β-cell islets and increasing insulin release in diabetes-induced rats (Vessal, Hemmati, Vasei, 2003).

CHAPTER FIVE

5.0 SUMMARY

In summary, the study found out that the avocado polyphenolic peel extract contained the following phytochemicals; p-coumaric acid, quercetin, and kaempferol. Also, following the feeding of the supplemented diet to the alloxan-induced groups, observed a significant ($p < 0.05$) reduction in the blood glucose levels, high-density lipoprotein cholesterol (HDL-C), malondialdehyde level, Homoestatic model assessment score Insulin resistance (HOMA-IR), atherogenic index, coronary artery index, and serum insulin levels and increased levels of total protein, SOD, CAT, as well as NO.

5.1 CONCLUSION

Conclusively, the diet revealed more anti-diabetic properties activity at 100 mg/kg and 200 mg/kg been the best of all groups that was fed. The anti-diabetic activity may be ascribed to the phytochemicals present, did not produce any dangerous effect on the parameters as well as the wellbeing of the experimental animals that was use for the study. Hence, avocado polyphenolic peel extract supplemented diet could be said to be safe for consumption and be used in the pharmaceutical industries or food industries.

5.2 RECOMMENDATION

1. The safety assessment and investigation of the possible mechanism of action of the anti-diabetic principles of *P. americana* polyphenolic peel extract can be further looked into.
2. The isolation of the beneficial compound found in the polyphenolic extract of the peel, as well as its application in pharmaceutical and food industries.

5.3 CONTRIBUTION TO KNOWLEDGE

The study was able to deliver scientific evidence regarding the use of the polyphenolic peel of *p. americana* as a supplement in the management of diabetes.

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APPENDIX

Preparation of 0.25 M Sucrose solution

85.5 g sucrose was dissolved in small quantity water and made up to 1 litre with distilled water.

Serum Total Protein Determination Reagents preparation

Biuret

1.5 g of copper sulphate ($CuS0_4.5H_20$) and 6.0 g of sodium potassium tartarate ($NaK.C_4H_4O_6.4H_2O$) were dissolved in distilled water to make 500 ml solution (solution A). 3 g of sodium hydroxide (NaOH) was dissolved in distilled water to make 300 ml Solution B was then added slowly to solution A with continuous stirring and the resulting solution transferred to a one-litre standard flask and the volume made up to the marked level with distilled water. The solution was stored in a polythene bottle.

Reagent for Reduced glutathione

This method has the following advantages over the earlier modification of the nitroprusside method.

(i) The precipitation process is carried out with a single easily prepared reagent. It does not required addition of solid sodium chloride or prolonged

(ii) The determination may be carried out at any temperature likely to be encountered in the laboratory.

(iii) The colour formed is relatively stable

(iv) The reagent for colour development is stable for many weeks

(v) The sensitivity of the method is so great that it may readily be adapted to a micro procedure.

Reagents

Glutathione Working Standard:

40mg of reduced glutathione (Sigma Chemical Co. London Mol. Wt= 307.3) was dissolved in 0.1M phosphate buffer, pH 7.4 and the volume made up to 100 ml with the same.

0.1M Phosphate buffer, pH 7.4

(i) 0.1M $Na_2HPO_4.12H_2O$ (Mol. Wt =358.22) was prepared by dissolving 7.16 g in 200 ml of distilled water.

(ii) 0.1M $NaH_2PO_4.2H_2O$ (Mol.wt -156.03) was prepared by dissolving 1.56 g in 100 ml of distilled water.

(iii) 0.1M phosphate buffer was prepared by adding 200 ml of solution (i) to 100 ml of solution (ii) and the pH was adjusted to 7.4 with drops of concentratedHCl or NaOH as the case may be.

Ellman's Reagent (5′5′-Dithiobis –(2-nitrobenzoate) –DTNB

40 mg (0.4g) of DTNB was dissolved in 0.1M phosphate buffer, pH 7.4, and the volume made up to 100 ml.

Precipitating Reagent

4% sulphosalicyclic acid ($C_7H_6O_6S.2H_2O$. mol.wt = 254.22) was prepared by dissolving 4 g of sulphosalicyclic acid in 100 ml of distilled water. Diluted precipating reagent is obtained by mixing 2 ml of phosphate buffer, pH 7.4 with 3ml of precipitating agent.

Calibration of GSH standard Curve

Serial dilutions of the GSH working standard were prepared as shown in the table below and GSH concentration was proportional to the absorbance at 412 nm. The readings were taken within 5 minutes. This is because the colour is stable for at least 5 mins after the addition of Ellman's reagents. A graph of absorbance against concentration was plotted.

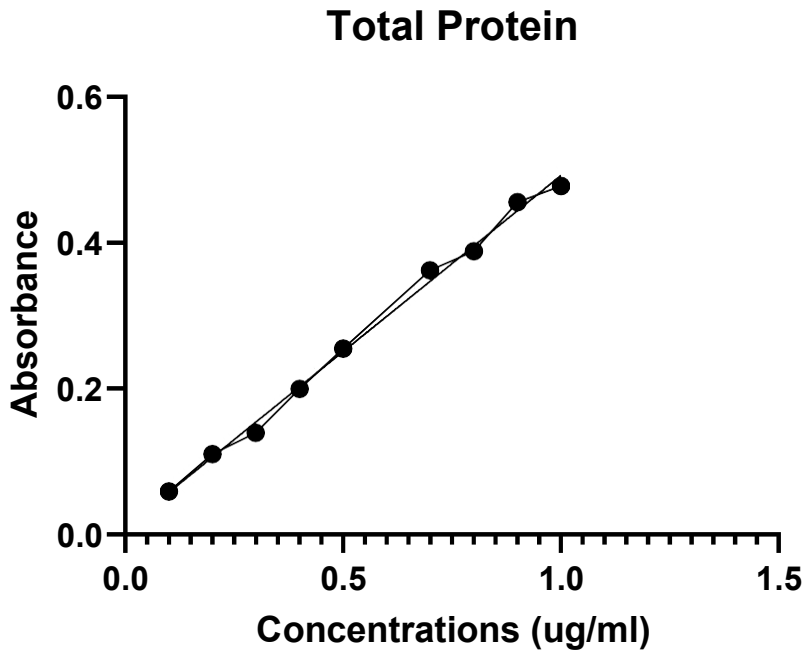

Figure 17: Calibration curve for total protein determination

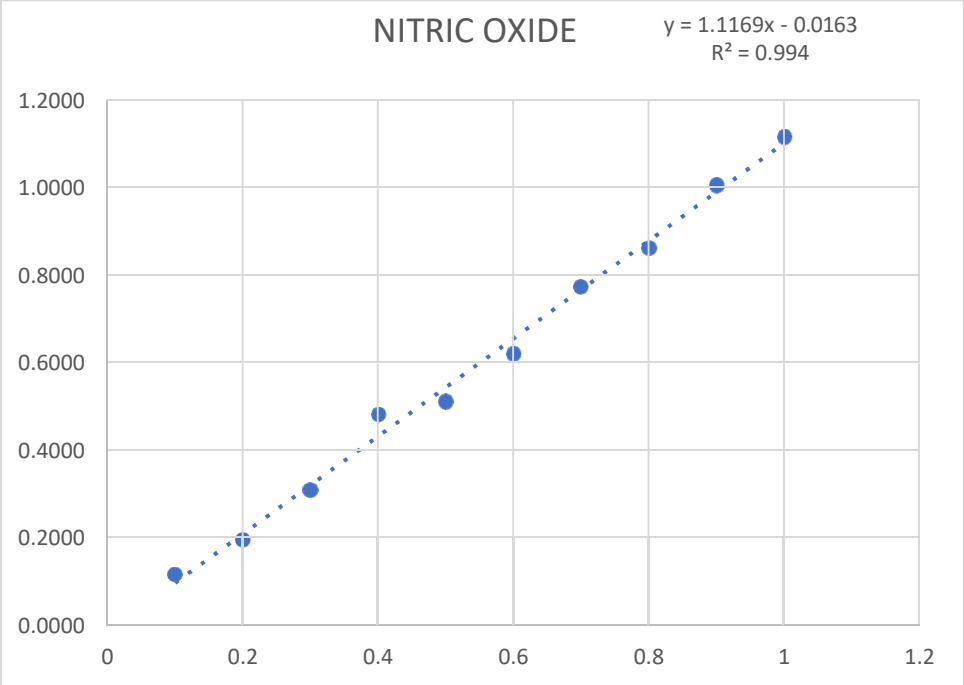

Figure 18: Calibration curve for Nitric oxide determination

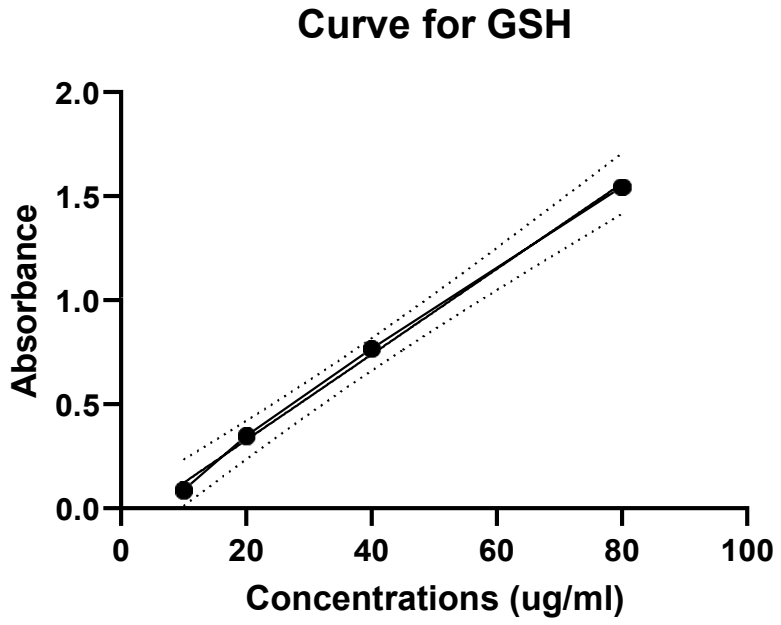

Figure 19: Calibration curve for GSH determination

Table 11: Total protein concentration readings

Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
0.21	0.087	0.156	0.111	0.172	0.105
0.194	0.101	0.106	0.11	0.174	0.104
0.202	0.094	0.131	0.108	0.173	0.103
0.214	0.88	0.157	0.176	0.197	0.158
0.18	0.101	0.105	0.175	0.2	0.153
0.197	0.095	0.131	0.175	0.201	0.15
0.215	0.089	0.157	0.144	0.186	0.132
0.178	0.1	0.105	0.143	0.187	0.129

Table 12: Catalase activity readings

Time (Sec)	Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
0s	0.110	0.179	0.15	0.125	0.174	0.121
0s	0.113	0.169	0.152	0.122	0.173	0.126
0s	0.114	0.17	0.153	0.124	0.175	0.106
30s	0.107	0.184	0.147	0.121	0.17	0.156
30s	0.105	0.183	0.146	0.119	0.176	0.155
30s	0.103	0.184	0.144	0.12	0.175	0.157
60s	0.098	0.175	0.14	0.118	0.157	0.136
60s	0.097	0.176	0.145	0.115	0.154	0.138
60s	0.095	0.174	0.147	0.117	0.156	0.136
90s	0.093	0.163	0.135	0.113	0.142	0.122
90s	0.093	0.165	0.133	0.109	0.146	0.12
90s	0.094	0.164	0.134	0.11	0.143	0.123
120s	0.088	0.159	0.129	0.106	0.139	0.1
120s	0.089	0.158	0.127	0.107	0.136	0.099
120s	0.087	0.159	0.128	0.105	0.14	0.11

Table 13: Superoxide dismutase activity readings

Time (Sec)	Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
0s	0.09	0.05	0.08	0.10	0.08	0.08
0s	0.08	0.05	0.08	0.09	0.08	0.08
0s	0.08	0.06	0.08	0.09	0.08	0.08
30s	0.08	0.05	0.08	0.09	0.08	0.08

30s	0.09	0.05	0.08	0.09	0.08	0.09
30s	0.09	0.06	0.08	0.09	0.08	0.08
60s	0.08	0.05	0.08	0.08	0.08	0.08
60s	0.10	0.06	0.09	0.09	0.09	0.09
60s	0.09	0.06	0.08	0.09	0.09	0.09
90s	0.10	0.05	0.08	0.09	0.09	0.08
90s	0.09	0.05	0.09	0.10	0.09	0.08
90s	0.08	0.06	0.09	0.09	0.08	0.08
120s	0.08	0.05	0.09	0.09	0.09	0.08
120s	0.09	0.06	0.09	0.09	0.09	0.09
120s	0.09	0.06	0.09	0.09	0.09	0.09
150s	0.10	0.06	0.09	0.09	0.09	0.08
150s	0.10	0.06	0.08	0.09	0.12	0.10
150s	0.09	0.06	0.09	0.10	0.08	0.09

Table 14: Body weight data of diabetic male rats before Induction

Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
183.07	362.36	203.98	265.45	270.46	253.89
172.06	279.02	211.46	220.35	288.82	278.24
201.83	351.78	157.87	217.23	242.83	350.72
187.44	233.98	181.26	250.33	218.92	325.57
207.01	285.24	201.98	213.59	263.83	220.59
133.02	346.21	188.8	168.12	322.32	300.6

Table 15: Body weight data of diabetic male rats after 7 days

Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
175.91	153.26	319.7	191.48	267.7	324.8
184.9	187.23	167.41	261.24	298.75	239.36
125.92	150.26	168.76	211.93	195.6	181.43
204.25	168.54	194.08	256.6	322.22	319.67
188.62	153.26	319.7	191.48	267.7	324.8
211.34	187.23	167.41	261.24	298.75	239.36

Table 16: Body weight data of diabetic male rats after 14 days

Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
202.37	153.26	169.79	257.42	312.08	331.76
133.4	187.23	182.32	183.99	190.45	310.49
192.56	163.25	212.5	283.84	286.85	231.56
212.18	153.26	169.79	257.42	312.08	331.76
227.32	187.23	182.32	183.99	190.45	310.49
184.74	163.25	212.5	283.84	286.85	231.56

Table 17: Body weight data of diabetic male rats after 21 days

Normal control	Diabetic Control	Standard drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
242.54	187.23	224.46	251.98	304.57	228.45
143.24	148.2	154.18	211.14	327.94	299.78
227.75	142.6	179.73	308.76	136.63	350.23

201.86	187.23	224.46	251.98	304.57	228.45
199.91	148.2	154.18	211.14	327.94	299.78
233.63	142.6	179.73	308.76	136.63	350.23

Table 18: Fasting blood glucose readings before induction

Diabetic Control	Standard Drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
106.2	90	99	95.4	88.2
90	84.6	88.2	86.4	113.5
91.8	88.2	95.12	86.4	100.85
106.2	90	99	95.4	88.2
90	84.6	88.2	86.4	113.5
91.8	88.2	95.12	86.4	100.85

Table 19: Fasting blood glucose readings after 72 hours

Diabetic Control	Standard Drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
547.25	304.2	532	541	397.8
534	475.2	397	486.5	416.4
452.1	415.5	268.2	513.75	374.4
547.25	304.2	532	541	397.8
534	475.2	397	486.5	416.4
452.1	415.5	268.2	513.75	374.4

Table 20: Fasting blood glucose readings after 7 days

Diabetic Control	Standard Drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg

365	300.6	280.8	269.32	253.8
482.3	358.24	374.4	275.06	394.2
296	324.15	396	280.8	329.4
482.3	358.24	374.4	275.06	394.2
296	324.15	396	280.8	329.4

Table 21: Fasting blood glucose readings after 14 days

Diabetic Control	Standard Drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
487.23	100.4	235	152.07	104.4
471.6	279	261.25	200.86	259.2
512	250.79	225	212.4	334.8
471.6	279	261.25	200.86	259.2
512	250.79	225	212.4	334.8

Table 22: Fasting blood glucose readings after 21 days

Diabetic Control	Standard Drug	Diabetic + 50 mg/kg	Diabetic + 100 mg/kg	Diabetic + 200 mg/kg
365	97.2	120.23	88.2	100.5
408.7	169.2	223.2	154.8	220.41
450.06	201.6	235.87	149.4	234
408.7	169.2	223.2	154.8	220.41
450.06	201.6	235.87	149.4	234

CONVERSION FACTOR

*HOMA-IR = [Insulin (U/l) × Blood glucose (mmol/l) / 22.5]

*HOMA- β = [20 × Insulin (U/l)] / [Blood glucose (mmol/l) – 3.5]

Conversion factor: Insulin (1U/l = 7.174 pmol/l) and blood glucose (1 mmol/l = 18 mg/dl).