IN VITRO AND *IN VIVO* ANTIDIABETIC ACTIVITY OF 70 % ETHANOLIC FRUIT EXTRACT OF *Rosa abyssinica* R.Br. ex Lindl ***(Rosaceae)***

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This is to certify that the dissertation prepared by Mohammed Ahmed Abdu, entitled: '*In vitro* and *in vivo* antidiabetics activity of 70 % ethanolic fruit extract of *R. abyssinica*'' and submitted in fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

In vitro and *in vivo* antidiabetic activity of 70 % ethanol fruit extract of *Rosa abyssinica*

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Diabetes Mellitus (DM) is a metabolic disorder result from defects in insulin secretion, action or both. Given the high prevalence of disability and comorbidities associated with DM, it's pivotal to bring forth sensible preventative and alternative treatment plans. Many plants in Rosacea family have been studied for their antidiabetic activity and *Rosa abyssinica* is one of the plant that widely used for its antidiabetic activity in traditional medicine in Ethiopia. The purpose of this study was to assess antihyperglycemic outcome of 70% ethanol extract of *Rosa abyssinica*. The *in vitro* antidiabetic effect was evaluated using assay for α-amylase inhibition of 70% ethanol fruit extract of *R. abyssinica* and positive standard, acarbose, at six different concentrations using 3,5-dinitrosalicylic acid (DNSA) technique. Conversely, normoglycemic, glucose-loaded, and streptozotocin (STZ)-induced diabetic mouse models were used to assess the *in vivo* antihyperglycemic activity. Five groups of mice—six mice in each group—were used in this investigation, consists of three experimental groups receiving 100, 200, and 400 mg/kg of the extract, positive control group receiving glibenclamide (GLC5 mg/kg), and negative control group receiving distilled water (10 ml/kg). In STZ-induced diabetes, a single intraperitoneal injection of 180 mg/kg body weight of STZ was used to cause diabetes. Anti-hyperglycemic effect of the extract in STZ - induced diabetic mice was evaluated using single dose and repeated dose study for three weeks. The *in vitro* test for α-amylase inhibition was analyzed using independent sample t test and the result showed there were no significant difference between the extract and the standard drug, acarbose, with triplicate measurement of IC50 of 26.72 ± 3.60 and 21.37 ± 4.25µg/ml respectively. The *in vivo* antihyperglycemic effect both in the oral glucose challenge and STZ induced diabetic mice showed similar result between the positive control and the two highest dose of the extract. In the normoglycemic experiment except with the highest dose of the extract, RA400, the other doses of the extract showed no hypoglycemic side effect and the extract showed positive for all tested qualitative secondary metabolite tests and quantitative test results showed alkaloids (83.37 mg ATP/g), phenols (892 mg GAE/g) and flavonoids (286.58 mg QCE/g) amounts.

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1. Introduction

1.1 Overview of Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disease brought on by an abnormality in the production, action, or combination of both of insulin. In turn, a lack of insulin causes persistent hyperglycemia and problems with the metabolism of fat, carbohydrates, and proteins [1]. Chronic hyperglycemia in turn results macro (i.e., coronary heart disease, hypertension, stroke) and microvascular complication (i.e. diabetic retinopathy, nephropathy, neuropathy) [2]. Approximately 5% of fatalities worldwide occur due to diabetes each year [3].

The Latin word “mellitus”, which literally translates as "honey" or "sweet," was first used by English physician Thomas Willis in 1675 to characterize the sweetness of a diabetic patient's urine. The Greek word “diabetes”, which means to go through or siphon, was first used by Araetus of Cappodocia (81–138 AD) to describe the polyureic nature of the disease and from these two words the term diabetes mellitus was drived [4]. A Liverpool physician Mathew Dobson (1735–1776) confirmed that diabetic patients had sugar in both their blood as well as in their urine and the excess sugar was not produced in kidney and the sugar was in fact was glucose was identified [4].

Claude Bernard identified this material, called it “glycogen”, and attempted to explain its fate in 1857 while explaining the patient's overproduction of glucose in the liver [5]. In 1947, Gerty and Corr were granted a Noble Prize for their discovery of how a phosphorylate enzyme, glucagon, catalytically converts glycogen into sugar [6].

Richard Bright (1831) and von Recklinhausen(1864) documented significant alterations in pancreas of patients with diabetics . Pancreatic islet cells were called after Paul Langerhans, who characterized their histology and gross anatomy in 1869 [4]. Joseph von Mering and Oskar Minkowsko made the great breakthrough in identifying the actual role of pancreases in pathogenesis of diabetes mellites by showing pancreatomized dogs acquired rapid and lethal diabetes and it was certain that pancreas plays a big role in the metabolism of carbohydrates, and the islets of Langerhans secrete an internal secretion [7]. The next challenge was to separate the internal secretion and the term "insulin," which comes from the Latin word for "island," was suggested by Edward Sharpy Shafer in 1916 for these compounds [4]. It took 30 years to find, isolate, and use insulin clinically, despite the fact that by the end of the 19th century, the significance of the pancreatic islets in the pathophysiology of diabetes had been recognized [4].

Insulin was discovered in 1921 by Fredrick Grant Banting, Charles Herbert Best and a biochemist James Collip. The study was one of the biggest millstones in history of medicine to work on isolation and purification of insulin extract, so as to be safe for human use and a new era in diabetes care and medicine has begun. In 1923, Banting and Best were granted the Noble Prize in medicine [8].

1.2 Types of Diabetes mellitus

Type one and two diabetes (T1D, T2D) classifications of diabetes mellitus have historically been gross oversimplifications of a scenario that is actually very complex and involves multiple overlapping effects from both heredity and environment. The categorization of T1D and T2D may be viewed as representing the extremes of a diabetes continuum, whereby the intermediate subtypes include maturity-onset diabetes of the young (MODY), latent autoimmune diabetes in adults (LADA), and more [9].

A. Type one Diabetes (T1D) - Also known as insulin-dependent diabetes, is defined by autoantibodies such as glutamic acid decarboxylase (GAD) antibodies, almost total absence of insulin production, the need for insulin injections and arises from the pancreatic islets' β-cells being destroyed by an autoimmune reaction [9]. The most common age groups for diagnosis are young adults, adolescents, children and it has strong genetically inheritance element as the occurrence is there >30% when both parents are affected, 6% when the illness affects either parent and only 0.4 % without a history of the illness in the family [10].

B. Latent Autoimmune Diabetes in Adults (LADA) -A fraction of persons with type 2 diabetes who have obtained a clinical diagnosis and are older than 35 years of age have been found to have pancreatic autoantibodies, which can react with non-specific cytoplasmic antigens including insulin in islet cells. Binge GAD antibody positive, being older than 35 at diagnosis, and delaying the initiation of insulin treatment for the first six to twelve months are the three primary indicators of LADA [11].

C. Maturity Onset Diabetes of the Young (MODY)- This type of DM inherited from either parent with the disease gene located on one of non-sex chromosomes, diagnosed before 25 years, manifest varied levels of malfunction in the beta cells and have more than ten distinct genes with clearly defined mutations [9]. Despite being identified early, the condition is moderate and does not worsen over the span of 40 years to require insulin [12].

D. Maternally inherited diabetes and deafness (MIDD)- Up to 1% of peoples with diabetic may be affected, but doctors frequently miss it. It results from an adenine to guanine replacement at position 3243 of the mitochondrial DNA representing the transfer RNA **Leucine** gene. Because the role of tRNAs is to deliver certain amino acids to the ribosome to facilitate the production of proteins, tRNA Leu's aberrant structure result in reduced tRNA function. Due to cellular energy deficit, the illness presents as MIDD in the organs with the highest metabolic activity, such as the cochlea and endocrine pancreas [13]. Mitochondrial DNA is only passed down from the mother (spermatozoa do not contain mitochondrial DNA), so MIDD indicates maternal transmission. Many patients exhibit neurological issues in addition to hearing loss, much like MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke) patients do, which is also result from similar mutation at mitochondrial DNA (mtDNA) [9].

E. Diabetes in infancy - Is characterized as having both temporary and permanent forms, and beginning from moment of birth or first half year. Neonatal diabetes has been linked to mutation in several different genes. (KCJN11, SUR1, GCK, INS, etc.) [14].

F. Gestational Diabetes mellitus (GDM) – It is a transient form of diabetes that exhibits during pregnancy as hyperglycemia—clearly not the same as overt diabetes—and disappears after delivery. GDM manifests when pancreatic beta cells are not able to offset pregnancy-related increased insulin resistance and still pathophysiology of the condition is still unknown. It is believed that GDM is caused by a confluence of environmental and genetic causative factors. Because GDM tends to cluster in families, the condition has been connected to a genetic predisposition [15].

G. Type two Diabetes (T2D) - A non-insulin-dependent diabetes It is caused by a reduction in insulin synthesis and an inability to withstand the effects of insulin [7,9]. 80%–90% of all cases of diabetes that have been documented are of the most common type, T2D. A complicated interaction of genetic, epigenetic, and environmental variables leads to type T2D. This type of diabetes arises when pancreatic beta cells are unable to generate enough insulin to counteract the rising insulin resistance brought on by fat accumulation. Individuals with T2D are those who do not meet the requirements for LADA, monogenic types of diabetes, T1D, or secondary diabetes. T2D is more commonly linked to aging and usually appears in those over 35 [16]. DM can also emerge as a result of pancreatic or other endocrine disorders and named Secondary Diabetes [9].

1.3 Epidemiology of Diabetes mellitus

The burden of non-communicable diseases (NCDs) is steadily rising, making them into significant health issues and diabetes mellitus is one of them [17]. No matter how different their economies, epidemiology, or demographics may be, non-communicable diseases, especially, diabetes mellitus are becoming more prevalent worldwide [18].

The international Diabetes Federation Atlas (IDFA) states that in 2022 there were 537 million or 10.5% of the global population affected with diabetes and this number is expected to rise to 784 million or 12.5% of the global population by the year 2045 [19]. According to WHO this number increased from 108 million or 4.5 % of the global population in 1980 [20]. In 2022 alone diabetes or diabetes related death believed to have claimed the lives of 6.7 million peoples [19].

With China, India, Pakistan and the USA topping the charts with 141, 75, 33 and 32 million people with DM in 2022, respectively. There were 24 million with DM in Africa, 62 million in Europe, 90 million in Southeast Asia, and 205 million (38.3% of total in the world) in the Western Pacific [19].

Despite of the high occurrence of DM worldwide there is still absence of proper diagnosis, access to healthcare and many peoples live with DM without knowing they have it or with minimal signs and symptom [19]. According to IDFA 2022 report Ethiopia is one of the countries in Sub Saharan Africa mostly affected accounting for 1.9 million people live with diabetes [19].

From the three major types of DM, T1D usually presents in early childhood, adolescents, and accounts for 5–10% of DM diagnosis and the most common T2D, accounts for 90–95% of DM diagnosed and continues to expand quickly in global scale [21]. Gestational DM also increases future risk of 7.4-fold those individuals to develop T2D [22].

1.4. Risk factors of Diabetes

A person's lifestyle, environment, or genetic characteristics that have been associated as a cause to a sickness based on an epidemiological data are thought to be diabetes risk factors [23]. It has long been recognized that not all individuals or populations are at equal risk of developing diabetes. T2D has been difficult to define, identify, and categorize over time, but research into the risk factors for diabetes has advanced more clearly [23]. Ethnicity, genetics, lifestyle, and/or a

combination of these factors are risk factors that are major in determining a person's risk to develop T2D [9].

Genetically predisposed with T2D susceptibility gene, like the CAPN10 gene on chromosome 10 [9], family history of being affected with the DM as indicated by nearly 100% occurrence of DM in identical twins, aggregation in families and ethnical communities all suggest strong support to this idea that genetics and family history has strong element as a risk factor [23]. According to the Framingham Offspring Study, compared to offspring without parental diabetes, those who had a single parent with diabetes had a 3.5-fold higher risk of developing T2D, while those who had two diabetic parents had a 6-fold higher risk [24].

As the prevalence of T2D rises with age and called "adult/maturity-onset" highlight that age has strong association with T2D as a risk factor and age 45 has been considered a key cut-off point for determining the prevalence of T2D [25] . However, because of the current lifestyle pattern that result in excess body weight and less physical activity, rates of T2D have concerningly higher in younger adults between the ages of 30-39 by 70% and by 40% among the age range between 40 and 49 [25].

Poor and unhealth lifestyle, physical inactivity, smoking, and unhealthy eating habits due to increase in the consumption of processed meat and sugar-sweetened beverages exacerbate the risk of developing DM [26]. Additionally, Higher BMI, waist-height ratio, waist-hip ratio, and waist circumference all suggest higher levels of intra-abdominal visceral fat. These characteristics also interfere with insulin metabolism by generating serum free fatty acids, which can cause T2D [27]. Lower educational and socioeconomic status are associated with higher levels of stress, which disrupts the neuroendocrine system and impairs endocrine function, limited access to healthcare facilities and they are more likely to adopt unhealthy lifestyle choices which makes them more prone to T2D [28].

Some medical conditions including gestational diabetes also increase the risk of developing T2D later in life [29]. Bing Prediabetic which manifested by Impaired Fasting Glucose (IFG), fasting blood glucose level between 110 and 125 mg/dl and Impaired Glucose Tolerance (IGT) : - 2-hour oral glucose tolerance test reading between 140 and 200 mg/dl indicates chronic insulin resistance and poses a highly significant risk to develop T2D [23]. Despite having a genetic predisposition, obesity and inactivity significantly worsen it [30]. In the initial phases of insulin resistance, beta cells managed to handle normal glycemic homeostasis by secreting insulin more but these techniques

of compensation will exhaust the beta cell's ability to secret insulin in the long run and progress to the onset of T2D [31]. Some medication also identified in inducing diabetes in those individuals with IFG and IGT i.e., glucocorticoids, thyroid hormone, diazoxide, β-adrenergic agonists, thiazide diuretic, pentamidine etc. can induce diabetes as well [3].

Disease with genetic cause associated with diabetes includes: Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome [3].Viral infections may also result in diabetes like: Congenital rubella virus infection, Cytomegalovirus infection, Coxsackie virus infection [3]. Endocrinopathies: Includes acromegaly, Cushing 's syndrome pheochromocytoma, glucagonoma and hyperthyroidism [3]. Diseases of the exocrine pancreas: Includes pancreatitis, pancreatectomy also result in DM.

1.6. Normal physiology of insulin secretion

1.6.1 Pancreases and Cells of the Islets of Langerhans

A sizable gland called the pancreas is situated retroperitoneally at the level of lumbar spine vertebra (Ll-L3) and it rests in the concavity of the duodenum on its right side, whereas on the left it extends to the kidney's hilus and touches the spleen anteriorly [33].

Pancreas is a multifaceted gland that contain both exocrine and endocrine elements in which the exocrine part is arranged in acini, a system of ducts that the pancreatic ducts, drain, collects and distributes the exocrine secretion products [34]. The endocrine glandular tissue contains islets of Langerhans, which are tiny cell clusters that make up the endocrine component [33].

The pancreas contains islands of endocrine cells called islets of Langerhans. In human islets, approximately 30% of the cells, α-cells, produce glucagon, 60% of the cells, β-cells, produce insulin, the remaining 10% are made up of cells that produce somatostatin (δ-cells), pancreatic polypeptide (γ- or PP cells), ghrelin(ε-cells) and these hormone producing cells are dispersed randomly around the islet cells [35].

One important mechanism for controlling blood glucose is the coordinated regulation of glucagon, the hormone that functions as an antagonist to insulin, and insulin secretion [35]. Glucagon secretion from α – cells are controlled by both paracrine and intrinsic processes as witnessed by, for instance, hyperglucagonaemia in T2D may be caused by the loss of the α -cells' internal regulatory mechanisms, which are mediated by glucose and amino acids, and extrinsic regulation, which are

mediated by insulin and zinc [36]. It has been suggested that the gut hormones Glucagon-Like Peptide-1(GLP-1) and Gastric Inhibitory Polypeptide (GIP) have significant role in controlling glucagon secretion [35]. The sympathetic and parasympathetic nervous systems control and innervate the islets of Langerhans, like hypoglycemic event stimulate glucagon secretion [37].

Insulin is produced and secreted by beta-cells, which is stored in secretory granules with zinc, in reaction to high blood glucose level and activation brought on by neurotransmitters produced in response to meal [35]. The presence of incretin hormones also promotes the release of insulin. However, Somatostatin, a hormone secreted by nearby δ -cells, together with epinephrine, galanin, ghrelin, leptin, and zinc ions all prevent the release of insulin [35].

Because the expression of both lactate dehydrogenase (LDH1) and the mono carboxylate transporter (MCT1) is suppressed, almost all glucose entering glycolysis proceeds into the Krebs cycle, and little to no glucose is converted to lactate. In this way the beta-cell can act as a glucose sensor and adjust insulin secretion to the plasma glucose level. These makes substrate supply regulates glucose metabolism rather than energy demand [38]. The principal constituency of glucose transporters, Glut 2, which has a high Km, insulin independent, and has a low affinity for glucose, facilitates the diffusion of glucose into the b-cell [39]. The first and rate limiting stage in the glucose-induced insulin secretion (GIIS) reaction, which is performed by the enzyme glucokinase (GCK; also known as hexokinase IV), is the phosphorylation of glucose [38]. An increase in blood glucose levels in beta-cells causes an increase in ATP produced by the Krebs cycle in the mitochondria. As a result, the ATP-Sensitive K^+ Channel (K_{ATP} channel) activity is inhibited, which result in membrane depolarization, electrical activity, and Ca^{2+} influx through voltage-gated Ca $^{2+}$channels in the b-cell, which lead to insulin exocytosis and release. The K_{ATP} channel is open at rest and is hyperpolarized in the absence of glucose [38].

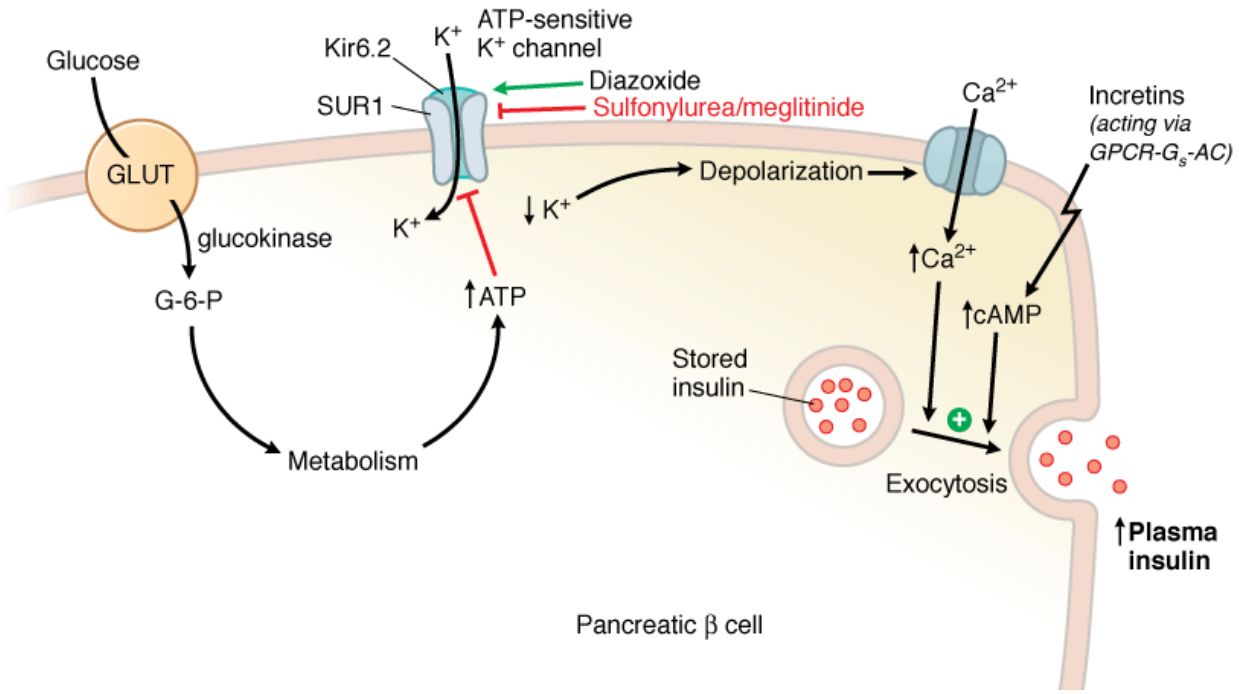

Figure 1: Regulation of insulin secretion from a pancreatic cell.

Resting pancreatic cells are hyperpolarized. Glucose, entering through GLUT2, is phosphorylated by glucokinase and enters the citric acid cycle and increases cellular ATP, inhibiting k^+ entry through the ATP-sensitive k^+ channel (K_{ATP}-Kir 6.2); the decrease in k^+ conductance leads to depolarization, leading to Ca^{2+}-dependent exocytosis of stored insulin. Incretins also improve insulin secretion [40].

δ cells or somatostatin secreting cells (SST-14) are present in pancreatic islets and also present in the hypothalamus, central nervous system, peripheral neurons and gastrointestinal tract [35]. Somatostatin is a negative regulator of insulin, glucagon and pancreatic polypeptide secretion under nutritionally stimulated and Ca^{2+}-dependent conditions [41]. Somatostatin binds to somatostatin receptor, which are G-protein coupled receptors and cause inhibition of adenylyl cyclase or activation of inwardly rectified K^+ channels [35,42].

One to two percent of the islet cell population are pancreatic polypeptide-containing cells, commonly known as PP cells or F-cells and they are more prevalent in the head of the pancreas. PP appears to have a satiety hormone-like action [35].

Islet ghrelin-positive cells - An orexigenic peptide called ghrelin increases appetite and the production of growth hormone (GH). It is mostly released from the stomach. In addition to modulating cardiovascular function, regulating food intake and energy metabolism, promoting osteoblast proliferation and bone formation, and stimulating myogenesis, neurogenesis and

performs a wide range of other tasks. Ghrelin has an impact on a number of processes in the digestive system, including pancreatic protein production, gastric motility, and acid secretion. The majority of these activities are thought to be accomplished by acylated ghrelin. The circulating level of ghrelin is determined by the equilibrium between its secretion rate, degradation rate and clearance rate [43].

1.7 Pharmacological Intervention of DM

Main categories of medications for DM includes insulin secretagogues (sulfonylureas and metiglinide - enhancer of insulin discharge from beta cells); biguanides (diminishes liver sugar production); peroxisome proliferator-enacted receptor-γ (PPARγ) agonists (Insulin activity enhancer); α-glucosidase blockers (disrupts absorption of sugar in the GIT) and furthermore Incretin mimetics (work like GLP 1 and GIP in animating insulin to be secreted by beta cells) and Sodium glucose co-carrier 2 blockers(inhibit glucose reabsorption at the kidney).

1.7.1 Insulin Secretagogues

These medications (particularly sulfonylureas and metiglinides) increase insulin secretion of beta cells by acting on sulfonylurea receptor (SUR), an ATP – sensitive potassium channel, on the beta cells [54].

The binding of sulfonylurea to SUR1 shuts ATP sensitive potassium channel, depolarizes the membrane of β–cells, open voltage dependent calcium channels and result in influx of Ca^{2+} into the beta cells which triggers exocytosis of insulin granules [55]. The first-generation sulfonylureas include, Tolbutamide, Chlorpropamide, Tolazamide, Acetohexamide and the second-generation sulfonylurea includes Glibenclamide, Glipizide, Glimepiride [56]. Development of second-generation sulfonylurea was due to increased potency, more rapid onset of action and longer duration of action. Sulfonylureas can cause side effects such as: dizziness, sweating, confusion, nervousness and also low blood sugar [57].

Metiglinides (glinides) are derivatives of benzoic acid of non-sulfonylurea moiety of glibenclamide. with lower affinity than sulfonylureas. These agents also exert their pharmacological effect by closing the ATP sensitive potassium channel found on pancreatic β cell ’s plasma membrane and some examples of this group includs Nateglinide, Mitiglinide, Repaglinide [58].

1.7.2 Biguanides

In contrast to insulin secretagogue, biguanides doesn't affect insulin secretion directly. By reducing hepatic gluconeogenesis, promoting glycolysis and by increasing insulin receptor's capacity to boost insulin signaling biguanides performs their antidiabetic activity [59]. Metformin improves the sensitivity and responsiveness of erythrocytes, adipose tissue, intestinal tract, and skeletal muscle to insulin. Without insulin, biguanide's sensitization function is ineffective. Metformin, Phenformin, and Buformin are examples of various compounds in this group. Due to the high prevalence of related lactic acidosis, phenformin and buformin were discontinued from clinical usage; however, metformin, which has a far lower risk, is still routinely used [59].

1.7.3 Insulin Sensitizers

Thiazolidinediones (glitazones) also called Peroxisome Proliferator Activated Receptor agonists (PPARs) which are regulators of protein and carbohydrate metabolism and control the balance of glucose [54]. There are the three subdivision of nuclear hormone receptor family that are ligand activated transcription factors, PPAR α, δ and γ. For the control of glucose, PPAR γ is specific [60]. Through PPAR activation, lipid reserves are transferred from extra-adipose to adipose tissue and circulating fatty acids are taken up by fat cells. Increased tissue sensitivity to insulin is one of the effects of coordinated cellular responses to PPAR activation, and this is the rationale behind the pharmaceutical use of thiazolidinediones in clinical medicine. Among the compounds in this group are pioglitazone, rosiglitazone, and ciglitazone [40].

1.7.4 Alpha Glucosidase and Alpha Amylase Inhibitors

By inhibiting carbohydrates hydrolyzing enzymes, α-glucosidase and α- amylase, present in the small intestinal brush border which are in charge of breaking down oligosaccharides and disaccharides into monosaccharides which are suitable for absorption, post-prandial glucose level management therapy for T2D could be carried out [61]. By pushing the undigested carbohydrate to the furthest part of small intestine and colon, these enzyme inhibitors decrease the process of glucose absorption in the GIT [62]. Alpha Glucosidase Inhibitors (AGIs) and Alpha Amylase Inhibitors (AAIs) are carbohydrates function as competitive inhibitors for the enzymes in the small intestine to minimize the digestion of carbohydrates, resulting in a decrease in postprandial

hyperglycemia [54]. Inhibitors of these enzymes includes Acarbose, Voglibose and Miglitol and other enzyme inhibitors used for management of T2DM [54,63].

1.7.5 Incretin mimetics

Naturally occurring metabolic hormones in the body known as incretins promotes a decline in blood glucose levels by promoting insulin release and decreasing glucagon [64]. Glucagon-like peptide (GLP), a 36 amino acid peptide, released by gut's L cells in response to meal intake and Glucose dependent Insulinotropic polypeptide (GIP) are naturally occurring peptides derived from gut [64]. These compounds cause beta cells to produce and secret insulin in respond to food intake [54]. However, due to its fast metabolism by the enzyme Dipeptidyl Peptide-4 (DPP-IV), GLP 1 has duration of action only about 1-2 minutes. As a result, it is necessary to produce GLP-1 analogues with longer half-lives as one method of managing diabetes [65].

1.7.5.1 GLP – 1 Agonist

DPP- IV easily metabolizes GLP-1 because of the presence of alanine, DPP-4's site of cleavage. Replacing this amino acid residue with others including threonine, glycine and serine result in new equivalents of GLP-1 with extended half-life and more resistant to DPP- IV metabolism [66]. Some examples of GLP-1 agonist include Exenatide, Liraglutide, Albiglutide, Dulaglutide and lixisenatide [65].

1.7.5.2 Dipeptidyl Peptidase - IV (DPP-IV) Inhibitors

A variety of physiologically significant peptides, including GLP-1, are degraded by DPP-IV. Consequently, DPP-IV inhibitors can increase the activity of these peptides. DPP-IV inhibitors include Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Alogliptin, Gemigliptin and Anagliptin [66,67].

1.7.6 Sodium Glucose Co-transporter 2 Antagonists/ Inhibitors

Reabsorption of glucose in proximal convoluted tubule (PCT) is achieved by passive transporter, facilitative glucose transporter (GLUT) or active sodium glucose co-transporter (SGLT) [54]. SGLT2 inhibitors inhibit the SGLT2 present in PCT which prevents reabsorption of glucose and enhances the excretion of glucose in urine. As glucose is excreted in urine, the glucose level in the blood is maintained and other glycemic parameters as well [68]. Some examples of drugs in this category includes Canagliflozin, Dapagliflozin, Empagliflozin and Tofogliflozin [69].

1.7.7 Insulin

Insulin has been available for the treatment of diabetes for almost a century, and the variety of insulin choices today represents many years of discovery and innovation. Insulin has gone from poorly defined extracts of animal pancreatic to pure and precisely controlled formulations manufactured using recombinant DNA technology that can be prescribed in rapid, short, intermediate and long-acting insulins, as well as mixtures and concentrated formulations and administered with high accuracy and predictability of action [70].

1.8 Non-Pharmacological Treatment Options in DM Management

Healthy dietary intake of nutrients and physical exercise contribute to normal plasma glucose levels in an individual. Therefore, nutritional therapies, lifestyle changes including increasing physical activity should be the focus of diabetes care [71]. With rising body weight, the risk of T2D climbs continuously and those who are morbidly obese are mostly at risk [72]. Overweighting with physical inactivity contribute to excess fat to accumulate mostly in the belly and visceral regions which result in decrement of insulin sensitivity and production of reactive free radicals which exacerbate diabetes [73].

The effective management of diabetes complication-related morbidity involve dietary therapies using dietary sources of antioxidants [74]. In order for T2D patients to obtain acceptable glycemic balance, nutritional therapies are essential and since most T2DM patients are obese, losing body weight by food prohibition helps in the management of DM [71].

During physical exercise, glycogen is consumed during the first few minutes of activity. However, if activity is sustained, the breakdown of muscle glycogen diminishes and reduction in blood sugar causes an increase in glucagon secretion and suppression of insulin release, therefore promote braking down of glycogen into glucose in the liver. When the exercise continued, muscle's glycogen reserves are exhausted and free fatty acids, which are produced when triglycerides are broken down, are utilized as a source of energy. By boosting GLUT4 translocation and expression, insulin sensitivity, lowering insulin resistance, and enhancing glucose disposal in peripheral tissues, exercise improves glucose uptake and usage in skeletal muscles [71].

To aid in weight loss, bariatric surgery involves making alterations to the digestive system. When exercise and nutrition doesn't work or the patient's weight is causing serious health problem, bariatric surgery is performed. The patient's capacity to eat is limited during some operations.

Other procedures operate by making it difficult for the body to take in nutrients. Some processes combine both [75].

1.9 Roles of traditional medicines in diabetes mellitus

Insulin and ranges of oral antidiabetic medications, including sulfonylureas, biguanides, α-glucosidase inhibitors and others are now the available treatment options for management of diabetes mellites. These antidiabetic medications currently are dearly expensive, hard to get in poor countries with rigged health care systems and the associated aftereffects of these medications result rise in considerable interest in herbal therapies. Therefore, utilizing plant- based traditional herbal remedies has become more pivotal in the management of DM [76]. Recently, herbal remedies have gained popularity and believed that more than 1000 plant species are utilized as traditional diabetic treatments [77].

Herbal products are abundant with secondary metabolites classified based on their unique structural characteristics includes, tannins, phenolic compounds, flavonoids, saponins, coumarins, terpenoids, alkaloids, terpenes, anthocyanin and these chemical compounds believed to be the plant's pharmacological activity arise from constitute reduction in blood glucose levels [78].

In scholarly and popular writing, a variety of traditional plant based organic medicine species have been determined to have antidiabetic qualities. Plant based remedies are recommended because they are fairly priced, believed to be beneficial and have fewer side effects in clinical settings. For management of diabetes mellitus, medicinal and herbal plant items have been utilized for a very long time in many different places [79].

1.9.1 Secondary Metabolites as Antidiabetics Agents

All plants include primary metabolites, which provide essential roles for the survival of the plants. Secondary metabolites, on the other hand, are organic substances generated from primary metabolites that support plant growth, development, adaptability and defense mechanisms but doesn't significantly contribute to the upkeep of plant life process. Their production may be ubiquitous or confined to certain families, genera, or even species, and they typically exist in relatively low quantities in specialized cells or tissue [80]. They also contribute to the specific Oduors, tastes and colors in plants, serving as attractants for pollinators, seed-dispersing animals and also contribute to signaling and the control of basic metabolic processes [80]. Diabetes was discovered to be significantly influenced by many plants' secondary metabolites [81]. It is known

that a number of phytoconstituents limits the action of intestinal enzymes involved in glucose absorption which result in disruption of glucose absorption at GIT and prevent postprandial hyperglycemia and maintain steady blood glucose level after eating [82]. Others can enhance the synthesis and release of glucagon-like peptide-1 (GLP1), restrain dipeptidyl peptidase-4 (DPP4) and extend the actions of GLP1 which encourage release of insulin and activate the hypothalamus gland to provoke satiety after eating and to block secretion of glucagon hormone [83]. By opening of calcium channel through inhibiting ATP sensitive K_{ATP} which results depolarization of the beta cells membrane and release of insulin, decreasing insulin breakdown by blocking insulin metabolizing enzyme or by blocking cAMP phosphodiesterase or other ways secondary metabolites might alter insulin secretion [84,85]. Secondary metabolites also protect beta cells from oxidative stress, reduce apoptosis, stimulate beta cells proliferation and enhance anti-oxidant capacity. [86,87].

1.9.2 *Rosa abyssinica*

Several ethnobotanical studies foretell numerous plant and herbs may have possible antihyperglycemic activity and out of which, *Rosa abyssinica* is one of them that being used in indigenous folk medicine for diabetes management in Ethiopia [88].

Rosa abyssinica belong to Rosaceae family and called "Kega" in Amharic, Qaqawwii in Oromiffa, Dayero in Somali and Abyssinian rose in English [89]. Rosaceae family includes 2,500 species and 90 genera and primely found in northern temperate region. Plants in this genus "*Rosa*" reported to have antineoplastic, anti-inflammatory and antioxidant, regulate blood sugar level, antimicrobial and antiviral activity, as well as nervous system and cardiovascular protective activities [90]. *R. abyssinica* remain green throughout the year with powerful, broad - based and curved prickles, sometimes forming a small tree of 0.5 - 7m of height with pale yellow fragrant flowers [89,91]. *Rosa abyssinica* found in Eritrea, Ethiopia, Sudan, Yemen and Saudi Arabia [92]. When the fruit ripens, the plant produces tiny, edible fruits that are crimson to brilliant orange in color and contain seeds [91,93].

Rosa abyssinica has great significance in Ethiopian traditional medicinal practice and used for management of several disease conditions including, rheumatoid arthritis, blood pressure, malaria, flu, scabies, tuberculosis and diabetes mellites [91,94,95]. It also claimed to have antimicrobial activity and also evaluated antidepressant, anti-inflammatory and interoceptive activity [96–98]. *Rosa*

abyssinica also have strong antidiabetic claims that being used extensively in Ethiopian traditional medicine after macerating with traditional alcoholic drink called "Araki" [88].

1.10 Statement of The Problem

Over the past few decades, non-communicable diseases (NCDs) including diabetes mellitus has emerged as one of the most urgent and widespread health challenge throughout the entire world [17].

Despite the promise of wide range options for prescription of antidiabetic therapeutics, these drugs are associated with numerous side effects, which are intolerable for many patients and the high cost of conventional medicines, easy availability and simple accessibility of traditional medicines, advice from friends, family and sometimes religious leaders led most of diabetic patients in the developing world including Ethiopia to use traditional medicines more often [99]. Certain people with diabetes think that taking both conventional and traditional medications together is more beneficial than taking them separately [99].

Given the high prevalence of disability and comorbidities associated with DM, it's pivotal to bring forth sensible preventative and alternative treatment plans [100]. Considering the scope of the traditional medicine usage of *Rosa abyssinica* for DM, lack of Publication, quality studies and specific scientific information about not efficacy but also safety beside its traditional claim and extensive usage makes this plant a candidate for this experimental study. This study, identification of *Rosa Abyssinia's* antidiabetic effect, done on crude extract of the plant, so further investigation of the plant with solvent fractionations is important to identify possible lead compounds for drug development against DM and the outcomes of this experiment will also serve as a model for future studies on the application of this plant.

2. Objective

2.1. General objectives

- To evaluate antidiabetic activity of 70% ethanol extract of fruit of *Rosa abyssinica.*

2.2. Specific objectives

- To evaluate *In Vitro* antidiabetic (α-amylase inhibitory activity) of 70% ethanol fruit extract of *Rosa abyssinica*.
- To evaluate oral acute toxicity of the extract in mice.
- To determine hypoglycemic effect of the extract in normal mice.
- To determine antihyperglycemic effect of the extract in glucose loaded normal mice
- To determine single and repeated dose antihyperglycemic effect of the extract in streptozotocin induced diabetic mice.
- To re-evaluate qualitative and quantify phytochemical constituents of hydroalcoholic extract *of Rosa abyssinica's* fruit.

3. Materials and Methods

3. 1. Drugs and chemicals

The preceding reagent, drug and material were utilized in the experiments. Streptozotocin (Sigma -Aldrich, St. Louis, MO, U.S. A), citric acid and trisodium citrate (Annexe Chem Pvt.Ltd, India), glibenclamide (Emcure Pharmaceuticals Ltd, India), 5% and 40% glucose (TNN Group, Dalian, Liaoning, China), distilled water, chloroform (Hely Specialty Chemicals, Ankleshwar, Gujarat, India). Mono and dibasic hydrogen phosphate (BDH Laboratory Supplies Ltd, England). Sodium chloride, sodium hydroxide, starch, potassium sodium tartrate tetrahydrate and α- amylase (Blulux Laboratories Pvt. Ltd., Faridaban, India). Bromocresol green solution and folin-Ciocalteu reagent (Ricca chemical, Arlington, TX-U.S.A) . Atropine, chloroform, gallic acid (Ralington pharma LLP,India), sodium carbonate , aluminum chloride , potassium acetate , quercetin(Tocris bioscience, Bristol-UK), ammonia, benzene, sulfuric acid , sodium nitroprusside , gelatin solution , lead acetate , potassium bismuth iodide(Fisher Scientific, UK), potassium mercuric iodide (May and Baker Ltd, England), 3,5-dinitrosalicylic acid (DNSA) (Sisco Research Laboratories Pvt. Ltd. Mumbai, India), acarbose (Bayer, Germany) and formalin (Genta Medical , UK). Almost all reagents, drugs and chemicals were analytical grade and purchased from their respective vendors.

3.2. Gathering of plant

Ripped fruit of *Rosa abyssinica* was brought from Majete, a town in north-eastern Ethiopia, 328 Km from Addis Ababa, located in North Shewa Zone, Amhara Regional state in September 2022. It was cleaned with tape water and let to air dry in the shade. Identification and authentication of the plant specimens was done by a taxonomist. A voucher specimen was deposited, voucher number: MA001, at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University.

3.3 Experimental animals

Swiss albino mice of both sexes, measuring 25-35 grams and aged 7-9 weeks, were acquired from the animal house of Ethiopian Public Health Institute (EPHI). All experiment and procedures on animal were carried out in compliance with the globally recognized guidelines for the use, care and welfare of laboratory animals as outlined in code of practice for the housing and care of animals used for scientific purposes [101] and ratified by department of pharmacology and clinical pharmacy research and ethics review committee by a letter with a reference number

ERB/SOP/466/14/2022**.** Prior to beginning the experiment, the mice were placed in animal house for a period of five to seven days in order to minimize the effects of environmental stressors on the immune, central nervous system, metabolism and endocrine systems. The mice were then randomly assigned to different groups [101]. Mice were kept in cages made of polypropylene, in groups of six, with bedding made of soft wood shavings that was changed every 24 hours, natural light exposure during the day, and the room was at room temperature. The mice were given unrestricted access to water both before and throughout the experiment, and when they weren't fasting, they were fed regular mouse pellets produced from pulverized animal food.

3.4. Extraction of Plant Material

The washed and dried fruit of *Rosa abyssinica* was grossly crashed with mortar and pestle into smaller pieces and dried for four weeks under shade and was powdered with blender. The resulted amount of powder, (746.44 g), was macerated for 3 days with 70 % ethanol (1 gram to 10 ml of solvent ratio) on auto shaker, to simulate traditional healer's usage, which they macerate it with traditional alcoholic drink called "Araki" [88]. And every 24hrs the mixture was filtered using gauze and Whatman filter paper No.1. The marc was re-macerated three times utilizing similar amount of ethanol to thoroughly remove the plant material. The filtrate from each procedure was collected in one place and ethanol was off from the extract by rotary evaporator and the water was removed using water bath which was fixed at 40ºc for 6 days and the final product 107.09 g (14.35 %) yield was produced, and the extract was kept in refrigerator and whenever required fresh stock solution was prepared.

3.5. Model, dose and groups

There were 152 mice in total used in this research: 6 for the acute oral toxicity study, 30 each for the oral glucose tolerance test (OGTT) and normoglycemic model, 80 for the Streptozotocin (STZ)- induced diabetes model, and 6 for the normal control group. The animals were divided into six groups at random and used to assess the hypoglycemic and antihyperglycemic activities in both in normal and stz-induced diabetic mice. The choice of dose was made using acute toxicity study**,** previous study on the plant extract [91,98] and also on the type of diabetes which was intended to be induced with streptozotocin, which was closer to T2D in this particular case with reduced number of beta cells. Considering variability exhibited by diabetes mellitus and different pathways a new agent might have pharmacological effect, it was beneficial to use some more animal models, and

these three in *vivo* models and one *in vitro* model were performed in this experimental study (i.e., normoglycemic, oral glucose loaded, STZ-induced diabetic and α amylase inhibitory activity models) [102]. Five groups of mice were randomly assigned each with six mice. Group I (negative control) received 10 ml/kg distilled water (DW), group II (Positive control) received the standard medicine glibenclamide (GLC5mg/kg) and groups III–V (test groups) received crude extract of *Rosa abyssinica's* fruit at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively after five days of acclimatization and fasting according to each model. The dose value for acute oral toxicity study was based on the acute oral toxicity guideline for OECD 425 [103]. Since sulfonylureas are known to enhance insulin production from preexisting pancreatic beta cells in the STZ-induced diabetic mice model, glibenclamide, a hypoglycemic medication, was selected as a reference drug [104,105]. The research was done utilizing oral route of administration to include the significant roles of intestinal hormones in the insulin release [102,106].

3.6. Blood glucose level measurement

In all animal models, blood samples were taken aseptically by cutting off the tip of the tail in order to measure the blood glucose level (BGL). Three BGL tests were performed using glucometer and the average result were used [115].

3.7. *In Vitro* antidiabetic effect study (Determination of α-amylase inhibition activity)

The 3.5- dinitrosalicylic acid (DNSA) technique was used to perform the α- amylase inhibition experiment [105,107]. Fruit extract of *Rosa Abyssinica* and positive standard drug, acarbose, were dissolved in a buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9). Different concentration in a range of 100 to 1000µg/ml of extract and acarbose were prepared by dilution method.

A volume of 200 µl of α-amylase solution, 2 units/ml, were mixed with 200 µl of each concentration of the extract and acarbose were incubated for 10 minutes at room temperature. After that, each tube received 200µl of the 1% starch in water (w/v) solution, which then incubated for three minutes. 200µl of DNSA reagent (12 g of sodium potassium tartarate tetrahydrate in 8 ml of 2M NaOH and 20ml of 96mM of 3,5-dinitrosalicylic acid solution) was added to end the reaction, and it was then boiled for 10 minutes at 87 °C in an oven. After that the mixture had reached room temperature, it was diluted with five milliliters of distilled water and a UV-visible spectrophotometer was used to measure the absorbance at 540 nm.

Absorbance of positive control (Ac) or absorbance of enzyme and buffer was prepared by replacing the plant extract or acarbose solution with 200 µl of buffer.

Absorbance of control blank, Acb (buffer without enzyme and inhibitor) was prepare by replacing inhibitors and enzyme with 200 µl of buffer; and each concentration of inhibitors (extract or acarbose) with enzyme or absorbance of sample, As (enzyme and inhibitor) and inhibitors without enzyme, absorbance of sample blank, Asb (inhibitor without enzyme) was prepared as the same procedure mentioned above.

The α-amylase inhibitory activity was expressed as percent inhibition and calculated using the equation given below [108].

Inhibition (%) =$((Ac-Acb) -(As-Asb))/((Ac-Acb)) * 100$

where Ac refers to the absorbance of positive control (enzyme and buffer); Acb refers to the absorbance of control blank (buffer without enzyme); As refers to the absorbance of sample (enzyme and inhibitor); and Asb is the absorbance of sample blank (inhibitor without enzyme).

Finally, the % α-amylase inhibition was plotted against the extract and acarbose concentration and linearity was obtained. Half maximal inhibitory concentration (IC50) value which is the concentration of sample required to inhibit 50% α-amylase activity was obtained from the graph for both extract and standard drug. Measurement was carried out three times for both, acarbose and extract, and the three IC50 value for each inhibitor was compared using SPSS (i.e., independent sample t test).

3.8. Acute oral toxicity study

Acute oral toxicity fruit extract of *Rosa abyssinica* was assessed using female Swiss albino mice, as per guideline of Organization for Economic Co-operation and Development, Guideline (OECD) 425 [103]. Five female, seven weeks old, Swiss albino mice were given an extract two hours after and four hours before administration of the extract, with water accessible at all time. The extract was prepared for the six mice and it was dissolved with distilled water in 2 ml of solvent /100g body weight of mice according to OECD. Each mouse was given volume of extract solution in line with their bodyweight. First, *Rosa abyssinica's* extract was given to a single mouse with a

2,000 mg/kg dosage and given that no fatality was seen, four more mice received an extract dosage of 2,000 mg/kg.

The animals were watched for four hours straight, with 30 minutes break during the first twenty-four hours. In addition to death, general sign and symptom of toxicity such as abnormal skin and fur color, tremor, convulsion, salivation, diarrhea and coma were seen. The observation was kept up for a total of 14 days. Finally, the animals were humanly killed on the 15th day.

3.9. Assessment of hypoglycemic activity in normal mice

Assessment of hypoglycemic activity in normal male mice was done as described elsewhere [109]. Five groups of mice were created, consisting of two control groups and three test groups. Each group included six male mice. The mice were given unlimited supplies of water during their overnight fasting. Glibenclamide(5mg/kg) was given to the positive control group, while distilled water(10mg/kg) was given to the negative control group and 100mg/kg,200mg/kg and 400mg/kg of *Rosa abyssinica* 70% ethanol crude extract was were given to the experimental categories. Administration was given orally via gavage, and the extract's effect were contrasted with those of the control groups. After glibenclamide, extracts and vehicle administration for 0, 1, 2, 3 and 4 hours, blood samples were taken from all groups. Using glucometer, the blood glucose level in mice's tail blood was determined [102].

3.10. Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) in normal glucose loaded mice was done as described elsewhere [106]. Male mice in good health condition were starved for fourteen hours with unlimited access to water. Following that, six mice per group were randomly assigned to one of five groups and mice's baseline(0hr) fasting blood glucose level was measured. Every mouse received 1,500 mg/kg glucose solution via orally half an hour after the extract of 100mg/kg ,200mg/kg and 400mg/kg of *Rosa abyssinica* was given and to the control group as given for the normoglycemic Mice. The amount of volume that were given from 40% glucose solution was calculated using the formula: Volume of glucose for injection (ml) = 0.00375 x body weight (g). Tail vein blood sampling technique with glucometer was used to measure blood glucose level before administrating distilled water, glibinclamide or extracts at (t=0) and 30, 60, 90 and 120 minutes after glucose delivery.

3.11. Induction of experimental DM

Streptozotocin (STZ) is a highly selective pancreatic islet β-cell cytotoxic medication that is commonly administered at a single high dose of 200mg/kg. It produces complete β-cell necrosis and diabetes with blood glucose reading more than 500mg/dl within 48 hours [110]. Even though literatures indicates that the kind of diabetes and its features vary according on the company, animal and species utilized, as well as the STZ dosage, experimental diabetes can be induced in male Swiss albino mice using streptozotocin in a single dose range of 150 mg/kg to 200 mg/kg [105,109,111,112]. Single i.p injection of STZ 180 mg/kg produced DM in the very first week and the animals remained in that state till the third week [111]. As a result, a single high dosage of 180 mg/kg STZ was used to induce diabetes. Eighty male mice, free to eat and drink, were acclimated for five days before the experiment began (female mice are less susceptible to the islet-cell toxin). On the first trial day, four hours before STZ administration, food was removed across all animal pens but water was provided as usual. First, STZ was dissolved in recently made 0.1M citrate buffer, which had its pH adjusted to 4.5 and the dose to be given was calculated using 180mg/kg. and given to the mice Intraperitoneal. Equal volume of citrate buffer, pH 4.5, was given to the normal control group mice using the same rout of administration. STZ was administered to each mouse immediately within 5 min of being dissolved [110].The mice were given normal food and 10 % sucrose water and they were attentively observed for 12 hrs to look for signs of hypoactivity, unresponsiveness, convulsions or any sign of fatal hypoglycemia. On the third day of the experiment, the 10% sucrose water was swapped out for normal water, and the mice were all fasted for six hours. The blood glucose level was then measured using a tail- vein blood sampling technique via glucometer and mice were classified as diabetics if their FBG level was 200mg/dl or higher [105,110] .

3.12. *Rosa abyssinica's* antidiabetic activity in STZ- induced diabetic mice

3.12.1 Single-dose study

Single-dose antihyperglycemic effect of 70% fruit extract of *Rosa abyssinica* extract was carried out 5 days after STZ injection on STZ-induced diabetic mice. Following a fourteen-hour fast (overnight fasting), blood sample was taken at 0, 1, 2, 3 and 4 hours-just before giving DW, standard drug (GLC5mg/kg) and three doses of extract (100mg/kg ,200mg/kg and 400mg/kg) of *Rosa abyssinica* as per grouping, 5 groups each with 6 mice [105].

3.12.2. Repeated doses study

Weekly antihyperglycemic effect of repeated dose of the extract was evaluated in STZ-induced diabetic mice. Diabetic mice, those utilized in the single-dose study, were given DW, glibenclamide and different doses of extract daily for 3 weeks. The effect of reducing blood glucose level of control, *Rosa abyssinica's* extract and glibenclamide was ascertained by taking a three-week measurement of the fasting blood glucose level every seven days after overnight fasting. FBG level of diabetic mice was measured first day, which was five days after STZ injection, and on the same time at the 1st, 2nd and 3rd weeks following fasting for 14 hrs. [105]. And also, for normal control (non-diabetic) mice distilled water and normal food were given for three weeks as well.

3.13. Determination of Body Weight

The effects of streptozotocin on body weight reduction and the enhancement of body weight change by extract and standard were identified. Prior to treatment, before STZ administration, the mice in the control group and all treated groups had their body weight measured and over the course of treatment i.e., 1st, 2nd and 3rd week of repeated dose study. The mice's body weight was measured using an electronics balance and the result was reported in gram.

3.14. Preliminary phtochemical examination

A preliminary qualitative phytochemical study was carried out on 70% ethanol *Rosa abyssinica's* extract using standard phytochemical reagents for the presence or lack of secondary metabolites by using conventional protocols including alkaloids, steroidal molecules, phenolic compounds, flavonoids, saponins, and tannins [98,113,114].

a. Test for alkaloids

- **Dragendorff's and / or Mayer's test -** 2 grams of the crude extract was placed in a test tube and mixed with 10 ml of 1% HCl for 30 min in a water bath and then filtered with Whatman filter paper No.1. the filtrate was divided into two test tubes. 1mL of potassium bismuth iodide solution (Dragendorff's reagent) and 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken in each. yellowish orange precipitate (for Dragendroff's reagent) and whitish / cream precipitate (for Mayer's reagent) indicated the presence of alkaloids.

b. Test for phenols

- **Ferric chloride test.** 1mL solution of an extract was taken and placed into a test tube. Then 1% gelatin solution containing sodium chloride was added and shaken. Formation of bluish-black color indicates the presence of phenols.

c. Test for flavonoids

- **Lead acetate test.** 1mL of extract was taken and placed into a test tube. Then five drops of lead acetate added and shaken. Formation of yellow precipitate indicated the presence of flavonoids.

d. Test for tannins

- **Gelatin's test.** 1mL of extract was taken and placed in a test tube. Then 1% gelatin solution containing sodium chloride added and shaken. Appearance of white precipitate indicates the presence of tannins.

e. Test for Saponins

- **Froth formation:** 0.5 g of 70% *Rosa abyssinica* crude extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for 30 sec The formation of "honey comb" froth that persisted indicated the presence of saponins.

f. Test for glycosides

- **Legals test.** 1ml of an extract was taken, and then an equal volume of sodium nitroprusside was added followed by few 3ml of sodium hydroxide solution and shaken. Formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside.

g. Test for steroids and triterpenoids

- **Salkowski's test**. 100 mg of 70 % ethanol extract of *Rosa abyssinica* were dissolved in 2 ml of chloroform, shaken, and filtered. Few drops of concentrated sulfuric acid were added to filtrate, shaken, and allowed to stand. Development of golden-yellow precipitate indicated the presence of triterpenes.

h. Test for free anthraquinones

- 100mg of the 70 % ethanol extract of *Rosa abyssinica* was shaken vigorously with 10 ml of benzene and the extract were filtered. The filtrate was treated with

5 ml of 10% ammonia solution and was shaken. The formation of pink/ red color in the ammonia phase was considered as positive for free anthraquinones.

3.15. Quantitative analysis for selected secondar metabolites

Secondary metabolites from fruit extract of *R.abyssinica* including alkaloids, flavonoids and phenols were selected for quantitative determination based on their capacity to restore pancreatic tissue function and potential antidiabetic activity [87].

A. Analysis of Total Alkaloid Content

Total Alkaloid Content (TAC) of 70 % ethanol extract of *Rosa abyssinica* was calculated using BCG, or bromocresol green solution, atropine standard solution and phosphate buffer with slight modification. This approach was based on the reaction of alkaloid with bromocresol green (BCG), forming a yellow-colored product, which easily extractable with chloroform at pH 4.7, that absorb maximum at 470nm [115–117]. A 1000 ml solution of bromocresol green solution was made by boiling 70 mg of bromocresol green, 3 ml of 2N NaOH, and 5 ml of distilled water until the BCG was completely dissolved. Disodium phosphate (4.543 gm Na_2HPO_4) and sodium dihydrogen phosphate (0.192 gm NaH_2PO_4) were combined with 1 L of distilled water to create phosphate buffer solution (pH 4.7. To create the atropine standard solution, 1 milligram of pure atropine was dissolved with 10 milliliters of distilled water. A series of concentrations (0(blank), 0.4ml (0.04mg/ml), 0.6ml(0.06mg/ml), 0.8ml (0.08 mg/mL), 1ml(0.1 mg/mL) and 1.2ml(0.12mg/mL) standard pure atropine dissolved in water was prepared and five milliliters each of phosphate buffer and BCG solution were added to each concentration, shaken vigorously and each concentration of standard atropine was adjusted the volume after being cleaned with 1, 2, 3, and 4 ml of chloroform and collected into a 10-ml volumetric flask . Using UV spectrophotometry, the absorbance was measured at 470 nm in comparison to the blank that was made as previously but without atropine. After dissolving 5 g of *Rosa abyssinica* sample extract in 2 N HCl until it was fully dissolved, Whatman filter paper No. 1 was used for filtering. After that, one milliliter of the filtered solution was put into a separatory funnel and three times rinsed with ten milliliters of chloroform, which was discarded, and pH of the solution in the separatory funnel was titrated to neutral Ph with 0.1 N NaOH. After that, the solution was mixed with 5 milliliters of BCG solution and 5 milliliters of phosphate buffer, and it was shaken. vibrant shaking was used to extract the residual complex using 1, 2 ,3 and 4 milliliters of chloroform. The extracts were collected in a 10-ml volumetric

flask and diluted it. At 470 nm, the complex's absorbance in chloroform was determined. The assay was performed in triplicate, with the mean value being determined.in milligrams of atropine per gram (mgATE/g), the results were given.

B. Analysis of Total Phenolic Content

Total phenolic contents (TPC) of 70% ethanol extract of *Rosa abyssinica* was determined using a Folin-Ciocalteu colorimetric method with slight modification[118]. Different concentrations of a standard phenol gallic acid solution (5, 10, 20,25,30,40 and 50 µg/ml) were prepared from (10mg of gallic acid/10ml distilled water) stock solution.7.5% (7.5 g/100ml) Sodium carbonate and a 10-time diluted Folin-Ciocalteu (FC) reagent were also prepared. 2.5 milliliters of purified water were poured into 1ml of diluted extract (5gram in 20 ml DW) and mixed with 0.5ml of FC reagent and incubated for 10 minutes before adding 2 milliliters of 7.5 % sodium carbonate was added, then kept it in the dark for 30 minutes. The resulted bluish colored solution's absorbance was determined at 765nm making a used of UV spectrophotometer. Identical processes were used for each gallic acid concentration, 2.5ml of DW,0.5 ml of FC, 2 ml of sodium carbonate was flushed and after the proper incubated time was over the resulted blue/greenish solution absorbance at 765nm was measured and the calibration curve was created. The test was run three times and the mean amount was recorded. The outcome was given as milligrams of gallic acid equivalents(mgGAE/g) per gram of extract.

C. Analysis of Total flavonoid Content

Total Flavonoid content (TFC) of 70 % ethanol extract of *Rosa abyssinica* was determined by Aluminum chloride colorimetric method as described elsewhere[119]. The principle of this method was that aluminum chloride form acid stable complex with the keto and/or hydroxyl groups in the flavonoids ring. Aluminum chloride (10% ,10g of aluminum chloride was dissolved in 100ml D.W), Potassium acetate (1M, 9.81 g potassium acetate was dissolved in 10 ml D.W) and Methanol was prepared. 15.62, 100, 200, 300, 400 and 500 µg/ml of standard quercetin solution was prepared from stock solution of (100 mg quercetin/100 ml) of methanol by dilution. 0.5 ml from each concentration of the standard solution were mixed separately in a test tube with 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate, and 2.8 mL of distilled water. 0.5 hr. later after incubation the absorbance of the solution was measured at 415nm against the blank prepared as above without quercetin and the standard curve with linearity was prepared. The same procedure

for the *Rosa abyssinica* fruit extract was followed. 1 ml from the prepared stock solution(100mg/100ml) was mixed with 0.1ml,0.1ml and 2.8ml of aluminum chloride, potassium acetate, and purified water respectively and the absorption was measured about 415 nm with ultra violate spectrophotometry against the control prepared as above without quercetin after 0.5 hr. incubation period at room temperature. The investigation was done three times and the mean value was recorded. The result was stated as milligrams of quercetin equivalent per gram of extract (mgQE/g).

3.3 Ethical principles

Experimental animals were cared for during the experimentation processes with care based on internationally recognized guidelines for the use, care and welfare of laboratory animals as set forth in Code of Practice for the Housing and Care of Animals Used for Scientific Purposes[101] which was a part of laboratory methods module. A copy of proposal was submitted to the respective committee and the research was conducted after obtaining approval and Ethical clearance from Research and Ethics Review Committee at Department of Pharmacology and clinical pharmacy, School of Pharmacy, Addis Ababa University (Ref. No. ERB/SOP/466/14/2022).

3.4 Data analysis

All statistical analyses were performed using international business machines of statistical package for the social Sciences, (IBM SPSS), version 26 for windows (SPSS inc, Chicago, Illinois, USA). For *in vitro* studies, independent sample t-test was employed. It was used to determine if there was or not statistically significant difference in α-amylase inhibition activity between extract and standard drug (Acarbose). For *in vivo* antidiabetic effect, statistical differences between groups and at different time was analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. In all cases results were expressed as mean ± standard error mean (SEM). P-values less than 0.05 were considered as statistically significant and P-values less than 0.01 and 0.001 were indicated to show the level of significance.

4. Results

4.1. *In vitro* α-amylase inhibitory activity

The *in vitro* α-amylase inhibitory assay of *Rosa abyssinica* showed remarkable inhibitory activity of the enzyme similar to the standard drug (Acarbose). IC50, which is the concentration of sample required to inhibit 50% α-amylase activity, was obtained from the adjusted concentration vs % inhibition linear graph. Figure 4 below shows how to calculate the IC50 of acarbose and extract of the first round of the experiment from the linear graph which was expressed as x-axis (concentration of acarbose and extract) vs y-axis (% inhibition). From the graph IC50 of acarbose and extract were 25.73µg/ml and 27.81µg/ml respectively and the R^2, which is a measure of the percentage of total variation in the % inhibition that is accounted by the acarbose or extract concentration[120], are 0.97 and 0.98 respectively. Whereas, the average IC50 value from triplicate measurements was 21.37 ± 4.252 µg/ml for acarbose and 26.72 ± 3.59 for the 70% ethanol fruit extract of *Rosa abyssinica* and all R^2 were above 0.9. The IC50 values which was obtained from each triplicate measurement of both extract and standard was compared with independent sample t test and the result showed there was no significant difference between acarbose and the extract in α-amylase inhibitory activity.

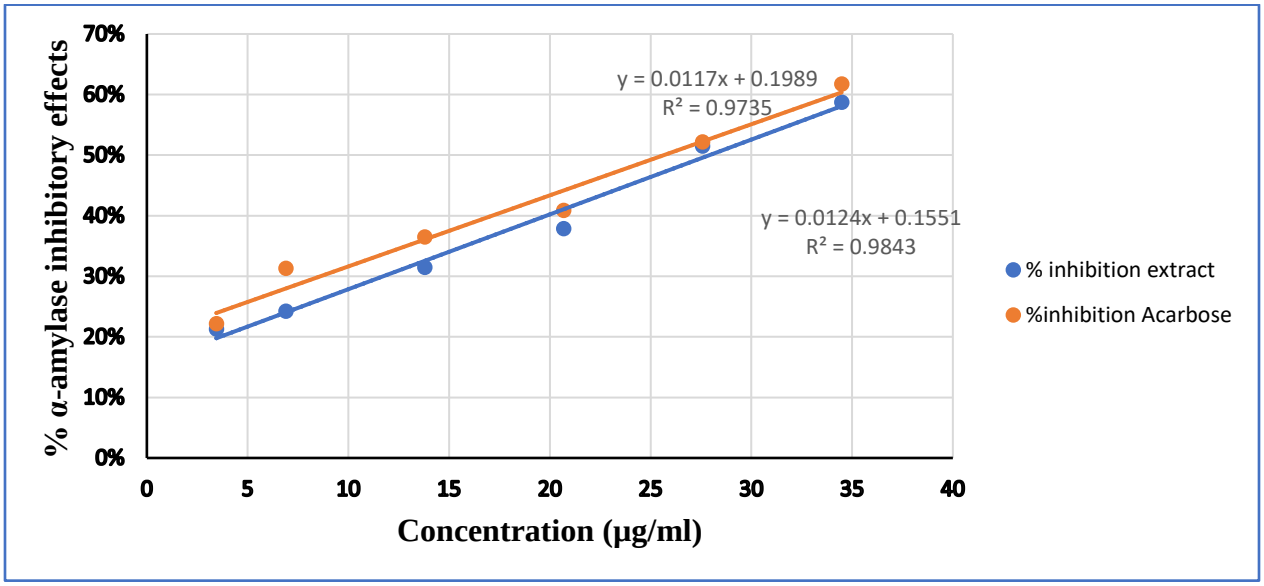

Figure 2: Concentration vs percentage α-amylase inhibitory effects of the 70% ethanol fruit extract of *Rosa abyssinica* and Acarbose

Table 1: Percentage inhibition and IC50 of α-amylasse of extract and acarbose

		Experiment 1		Experiment 2		Experiment 3	
Extract / Acarbose (µg/ml)	Final (µg/ml)	% Inhibition *R. abyssinica*	Acarbose	% Inhibition *R. abyssinica*	Acarbose	% Inhibition *R. abyssinica*	Acarbose
100	3.45	21.28%	22.17%	22.96%	37.06%	8.14%	15.08%
200	6.9	24.26%	31.30%	29.61%	47.50%	18.22%	28.57%
400	13.79	31.49%	36.52%	41.39%	42.59%	21.32%	36.51%
600	20.69	37.87%	40.87%	49.85%	62.54%	34.88%	40.87%
800	27.59	51.49%	52.17%	65.56%	68.38%	47.29%	51.98%
1000	34.48	58.72%	61.74%	69.79%	83.11%	50.39%	62.70%
IC50 (µg/ml)		25.73	27.81	12.87	20.03	25.52	32.32

4.2. Acute toxicity study

The oral route 2,000 mg/kg dose acute toxicity study of the 70% ethanol fruit extract of *Rosa abyssinica* showed no toxic effect on Swiss albino mice. After 24 hrs animals were found to tolerate the administration of the extract and showed no general signs and symptoms of toxicity, such as unusual skin and fur color, tremors, convulsions, salivation, diarrhea and coma as well as no mortality at the end of two week.

4.3. Hypoglycemic effects of 70% ethanol extract in normoglycemic mice

The hypoglycemic effects of 70% ethanol extract in normoglycemic mice are shown in Table 3.

Table 2: Effect of 70 % fruit extract of *Rosa abyssinica* on fasting BGL of normoglycemic mice

Group	Blood glucose concentration in (mg/dl)				
	0h	1h	2h	3h	4h
NC	109.50±7.297	108.17±7.373	105.33±7.446	102.33±6.917	101.33±7.701
GLC5	108.83±1.249	88.17±2.738$^{\bullet 1}$	82.00±1.506$^{\bullet 2}$	77.33±1.333$^{\bullet 3}$	72.33±2.060$^{\bullet 3}$
RA100	106.17±2.725	101.67±4.998	98.83± 4.512	92.17±2.676	87.50±3.547
RA200	106.83±4.556	98.50±1.765	95.33±1.892	90.00±2.490	84.67±1.647
RA400	106.50±9.369	91.00±2.658	88.67 ±1.116	86.33±1.022$^{\bullet 1}$	81.83±2.088$^{\bullet 1}$

Data are expressed as mean ± S.E.M; n=6 for each treatment; Analysis was performed by one way ANOVA; ●, compared to negative control; NC, negative control treated with distilled water; GLC, glibenclamide; RA, *Rosa abyssinica* extract; number followed by RA and GLC indicates dose/ in mg/kg; h, hour; 1p<0.05,2p<0.01,3p < 0.001.

The baseline fasting blood glucose levels (0h) of all groups showed no statistically significant difference between them (Table 5). But the positive control (GLC5) group showed highly significant (at 1hr $P<0.05$, 2hrs $P<0.01$, at 3 and 4hrs $P<0.001$) difference in reduction of blood glucose level compared with the negative control group. Except the RA400 which showed at 3 and 4 hrs. with ($P<0.05$) significant difference in reduction of blood glucose level compared with the negative control, none of the extracts dose produce any significant deference compared with the negative control group at all time points. In group analysis the extract treated groups showed no significant difference in reduction of blood glucose level compared with the positive control treated group and also with each other at all time points.

The blood glucose levels decline through time indicates there was reduction in all groups. However, the extent was less with the negative control and extremely high, even statistically significant with glibenclamide treated mice. However, none of the extract taking groups showed a significant reduction in BGL at all-time points

4.4. Antihyperglycemic effect of extract in oral glucose tolerance test

Table 4 below shows glucose lowering effect of extract in oral glucose tolerance test. There was no significant BGL difference among the negative control, positive control and extract treated groups at baseline (t=0h). Glucose loading produced hyperglycemia after 30 min of administration in all groups, with a maximum increase achieved with the NC (107.40%). However, only mice treated with the standard drug i.e., glibenclamide, 5mg/kg of body weight ($p<0.01$) and mice treated with the highest dose of the extract, RA400, ($p<0.05$) showed significant reduction in blood glucose level at 30 minutes as compared to NC group. Groups of Mice treated with 200mg/kg of extract at 60, 90 and 120 minutes and mice treated with 400mg/kg of extract at 60 minutes showed significant ($p<0.01$) reduction in blood glucose level compared with NC group. While mice treated with the highest dose of the extract at 90 and 120 minutes and mice treated with the positive control, glibenclamide 5mg/kg of body weight at 60, 90 and 120 minutes showed highly significant ($p<0.001$) reduction in blood glucose reduction as compared to negative control group.

Table 3: Effect of 70% ethanol fruit extract of *Rosa abyssinica* on oral glucose tolerance test

Group	Blood glucose concentration in (mg/dl)					% BGL increment
	0 Minute	30minuts	60minuts	90 minutes	120 minutes	At 30 minutes
NC	110.17 ± 1.662	228.50± 7.974	175.17± 2.182	163.83± 1.327	126.67±2.186	107.4
GLC5	106.50 ±0.885	173.67± 5.200$^{\bullet 2}$	113.17 ±3.311$^{\bullet 3}$	97.83 ± 4.045$^{\bullet 3}$	72.83±1.956$^{\bullet 3}$	39.59
RA100	106.17 ± 0.833	213.00± 16.777	170.83 ± 1.276	155.50±5.494	121.83±1.851	84.92
RA200	104.50 ± 2.156	194.83± 0.477	160.83± 2.182$^{\bullet 2}$	143.17± 1.302$^{\bullet 2}$	114.33±2.860$^{\bullet 2}$	84.52
RA400	107.83 ± 2.857	186.17±1.869$^{\bullet 1}$	160.33 ± 1.202$^{\bullet 2}$	112.00± 3.445$^{\bullet 3}$	105.83±2.386$^{\bullet 3}$	80.68

Data represents mean ± S.E.M; n=6 for each treatment; Analysis was performed by one way ANOVA; ●. compared to negative control; NC, negative control treated with distilled water; GLC, glibenclamide; RA, *Rosa abyssinica* fruit extract; number followed by RA and GLC indicates dose in mg/kg; 1p< 0.05; 2p< 0.01; 3p < 0.001.

4.5. Induction of experimental T2D in mice

From 80 male mice that were given 180mg/kg STZ for induction of diabeties, after 3 days 46 of them (with STZ's 57.5% induction rate of DM) had blood glucose level over 200 mg/dl. 1 animal died by the time of STZ administration, 2 animals died on the next day and 22 animals died in between the day of STZ administration and the 3rd day and 9 animals had between 150 –200 mg/dl of fasting blood glucose level. Out of 46 diabetic animals 30 of them were randomly selected and grouped into 5 groups (6 mice each) and single and repeated assays were performed.

4.6. Effects of administration of extracts to Streptozotocin -induced diabetic mice

4.6.1. Single dose Antihyperglycemic effect

The antihyperglycemic effects of single dose of extract on Streptozotocin -induced diabetic mice is shown in Table 5.

Table 4: Antihyperglycemic activity of Single dose 70% ethanol fruit extract of *Rosa abyssinica* in streptozotocin-induced diabetic mice

Group	Blood glucose level concentration in (mg/dl)				
	Baseline	1hr	2hrs	3hrs	4hrs
NOC	99.50±2.778	97.33±1.838	94.17±2.509	91.33±2.216	89.50±2.895
NC	364.83±48.11^{*3}	359.5±44.622^{*3}	348.00±40.899^{*3}	335.33±41.175^{*3}	321.83±43.411^{*3}
GLC5	380.50±12.92^{*3}	203.17±8.716$^{\bullet 1}$	111.17±3.291$^{\bullet 2}$	48.50±8.011$^{\bullet 3}$	41.33±7.619$^{\bullet 3}$
RA100	413.00±24.54^{*3}	335.33±7.566	301.17±3.135	274.50±7.549	243.67±10.806
RA200	382.17±36.98^{*3}	276.83±4.415$^{\bullet 1}$	176.67±5.590$^{\bullet 2}$	130.33±2.418$^{\bullet 3}$	106.17±2.574$^{\bullet 3}$
RA400	383.33±10.27^{*3}	245.33±7.714$^{\bullet 1}$	139.67±3.313$^{\bullet 2}$	100.50±2.604$^{\bullet 3}$	89.17±2.701$^{\bullet 3}$

Data represents mean ± S.E.M; n=6 for each treatment; Analysis was performed by one way ANOVA; $^{\bullet}$, compared to negative control; *, Compared with the normal control; number followed by RA and GLC indicates dose/ in mg/kg; hr., hour; GLC, glibenclamide; RA, *Rosa abyssinica* fruit extract; 1p< 0.05; 2p< 0.01; 3p < 0.001.

Except the normal control group (non-diabetic mice with mean value of 99.5 mg/dl) all groups of mice after induction of diabetics or at baseline(t=0hr.) showed no significant FBG level difference between them, all with mean value > 300 mg/dl. Compared to the normal control group, negative control group had highly significant ($p<0.001$) blood glucose level with mean value >300 mg/dl at all-time points. Except at baseline (t=0hr.) the positive control (GLC5), RA200 and RA400 showed a significant reduction in BGL compared with the negative control group at (1hr. $p < 0.05$,2hrs $p < 0.01$,3 and 4hrs $p < 0.001$). Group comparison showed that GLC5($p < 0.001$, at all-time points), RA200 and RA400 (at 2,3 and 4hr, $p < 0.001$) showed highly significant difference in BGL reduction compared with RA100. RA100 failed to show any significant different result compared with the negative control group at all time points. The positive control, GLC5, and two of the highest doses of *Rosa Abyssinica* extract, RA200 and RA400, had no significant difference

between them at all points. In the group analysis, the trend over time in the reductions was consistent up to fourth with the two higher doses of extract and positive control group. A decline in FBG level was also recorded with the smaller dose of the extract, RA100, negative and normal control group but with a lesser degree.

4.6.2. Repeated dose Antihyperglycemic effect

Weekly FBG level was measured to determine the repeated dose effect of *Rosa abyssinica* in streptozotocin-induced diabetic mice and the result is indicated in table 6.

Table 5: Weekly antihyperglycemic activity of repeated dose 70% ethanol fruit extract of *Rosa abyssinica.*

Group	Weekly FBG concentration in (mg/dl)			
	Baseline	Week 1	Week 2	Week 3
NOC	99.50±2.778	100.50±1.33	104.33±1.45	103.33±2.42
NC	364.83±48.11^{*3}	380.67±46.88^{*3}	393.00±46.19^{*3}	395.83±46.05^{*3}
GLC5	380.50±12.92^{*3}	188.33±4.40$^{\bullet 3}$	117.17±10.14$^{\bullet 3}$	85.00±6.01$^{\bullet 3}$
RA100	413.00±24.54^{*3}	349±7.33	320.67±7.15	303.00±4.18$^{\bullet 1}$
RA200	382.17±36.98^{*3}	283.67±9.75$^{\bullet 1}$	205.67±8.83$^{\bullet 3}$	143.17±6.67$^{\bullet 3}$
RA400	383.33±10.27^{*3}	251.50±20.63$^{\bullet 2}$	137.33±13.76$^{\bullet 3}$	103.67±15.67$^{\bullet 3}$

Data represents mean ± S.E.M; n=6 for each treatment; Analysis was performed by one way ANOVA; *, compared to normal control; $^{\bullet}$, compared to negative control; number followed by RA and GLC indicates dose in mg/kg; NOC, normal control treated with distilled water; NC, negative control treated with distilled water; GLC, glibenclamide; RA, *Rosa abyssinica* fruit extract; 1p < 0.05; 2p < 0.01; 3p < 0.001.

All STZ induced diabetic mice initially(t=0hr.) showed no significant difference in FBG level between them. However, the negative control diabetic group at all time points and the other groups (GLC5, RA100,200 and 400) at baseline showed significantly higher ($p<0.001$) in FBG level compared to the normal control group of mice. Multiple comparison results of the ANOVA analysis showed that the positive control, GLC5, showed significantly ($p<0.001$) higher difference in reduction of FBG level at all time points compared with the negative control and with RA100 ($p<0.01$) and also at week one with RA200($p<0.05$). Two of the highest doses of the extract (RA200 and RA400) showed highly significant($p<0.001$) reduction in FBG level at week two and

three compared with the negative control of diabetic mice. Also in between extract's group comparisons RA400 showed significantly high ($p<0.001$) difference in reduction of FBG level compared with RA100 at week two and three. Also, RA400 showed significant reduction at week one ($p<0.05$) compared with RA100. RA200 also showed significant difference compared to RA100 in reduction of BGL at week two ($p<0.01$) and at week three ($p<0.001$). While the negative control group was diabetic at all-time points, GLC5 at 2nd, 3rd week and RA200, 400 doses of extract at 3rd week tend to bring the FBG level to the normal control level. Also, RA400 ($p<0.01$) at first week and RA100 ($p<0.05$) at third week showed significant reduction in FBG level compared with the negative control.

4.7. Effect of 70% ethanol extract of *Rosa abyssinica* fruit on mice body weight

The effect of 70% *Rosa abyssinica* fruit extract on body weight of diabetic mice is shown in Table 7.

Table 6: Effect of 70% ethanol fruit extract of *Rosa abyssinica* on weekly body weight change of diabetic mice.

Group	Body weight in gram			
	Baseline	Week 1	Week 2	Week 3
NOC	31.1333±0.70	31.800±0.55	32.300±0.56	32.950±0.55
NC	30.1333±1.48	24.533±0.38^{*2}	21.617±0.56^{*3}	18.467±0.24^{*3}
GLC5	28.1833±1.08	27.083±1.01	28.773±0.872$^{\bullet 3}$	28.183±1.11$^{\bullet 3}$
RA100	31.5833±1.98	27.483±1.67	24.350±1.37^{*3}	21.43 ±0.4551^{*3}
RA200	29.7500±1.38	26.750±1.35	27.500±1.21$^{\bullet 2}$	28.350±1.18$^{\bullet 3}$
RA400	31.5500±1.52	30.467±1.53	29.635±1.55$^{\bullet 3}$	30.717±1.33$^{\bullet 3}$

Data represents mean ± S.E.M; n=6 for each treatment; Analysis was performed by one way ANOVA; *, compared to normal control; $^{\bullet}$, compared to negative control; number followed by RA and GLC indicates dose in mg/kg; NOC, normal control, non-diabetic treated with distilled water; NC, negative control, diabetic treated with distilled water; GLC, glibenclamide; RA, *Rosa abyssinica* fruit extract; 1p < 0.05;2p < 0.01 ;3p < 0.001.

There was no significant body weight difference among all six groups initially(t=0). In the weeks followed, However, the NC showed significant ($p<0.01$) in week one and highly significant($p<0.01$) in week two and three in reduction of body weight compared with the NOC

group. Multiple comparison of the one-way ANOVA analysis showed that after two and three weeks the NC and RA100 showed significantly high ($p<0.001$) body weight reduction compared with the NOC. The NC, compared at two weeks with GLC5, RA400 and at three weeks with GLC5, RA200 and RA400, showed significantly high($p<0.001$) reduction in body weight. At week two and three RA100 showed significantly high ($p<0.001$) reduction compared with NOC.at week two NC also showed significantly high ($p<0.01$) reduction in body weight compared with RA200.

4.8. Preliminary phytochemical screening

The preliminary phytochemical screening of 70 % ethanol fruit extract of *Rosa abyssinica.* Showed the presence of almost all tested secondary metabolites as mentioned in Table 8.

Table 7: 70 % ethanol fruit extract of *Rosa abyssinica*'s secondary metabolites

Screened secondary Metabolites	Test reagents/methods	Absence/presence
Alkaloids	Dragendroff's / Mayer's test	presence
Phenols	Ferric chloride test	presence
Flavonoids	Lead acetate test	presence
Tannins	Gelatin's test	presence
Test for Saponins	Honey comb test	presence
Glycosides	Legals test	presence
Steroids/triterpenoids	Salkowsk's test	presence
Anthraquinones	anthraquinones	presence

4.9. Quantitative analysis for selected secondar metabolites

Quantitative analysis for secondary metabolites from fruit extract of *R.abyssinica* indicated total alkaloid content (TFC), total phenolics content (TPC) and total flavonoid content (TFC) of 83.37 mg ATE/g, 892 mg GAE/ g and 286.58 mg QCE/g dry weight of extracts respectively (Figure 11,12.13).

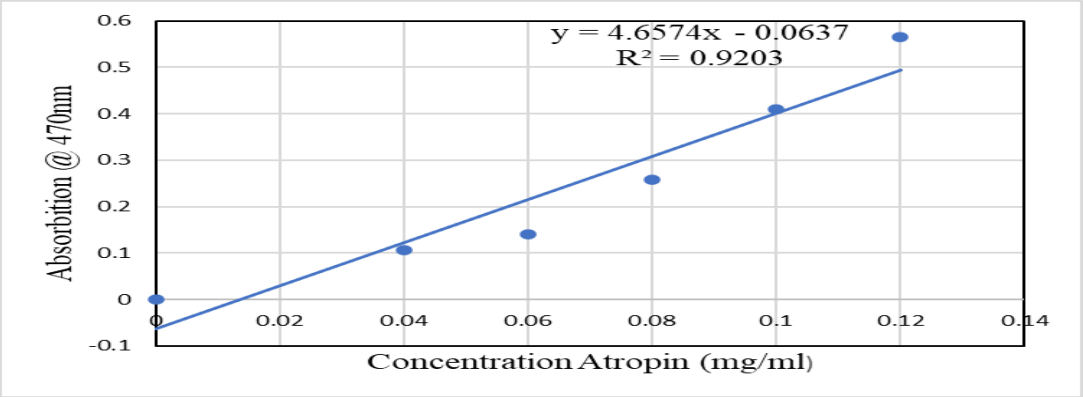

Figure 3: Atropine standard absorbance graph.

As shown on the graph above the standard absorbance graph for atropine was expressed in a linear line with the equation Y= 4.657X + 0.0637 and a correlation coefficient (R^2) of 0.9203. also, the average triplicate absorbance for the sample resulted in 0.452 and total Alkaloid content (TAC) 83.37 mg ATP/g dry extracts was calculated.

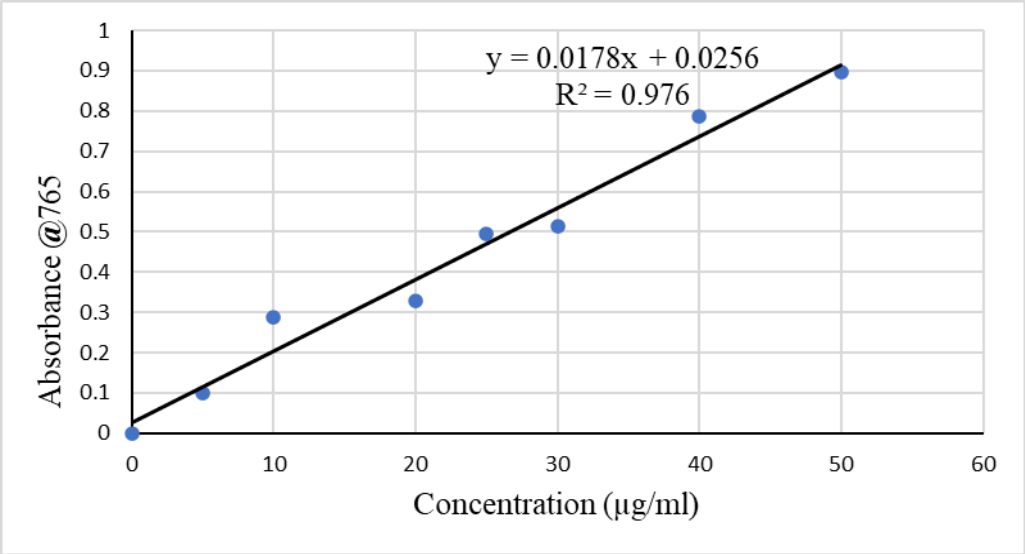

Figure 4: Gallic acid standard absorbance graph.

As shown on the graph above the standard absorbance graph for gallic acid was expressed in regration linear line with the equation Y= 0.0178X + 0.0256 and a correlation coefficient (R^2) of 0.976. also, the average triplicate absorbance for the sample resulted in 0.82 and total phenolic content (TPC) 432 mg GAE/g dry extracts was calculated.

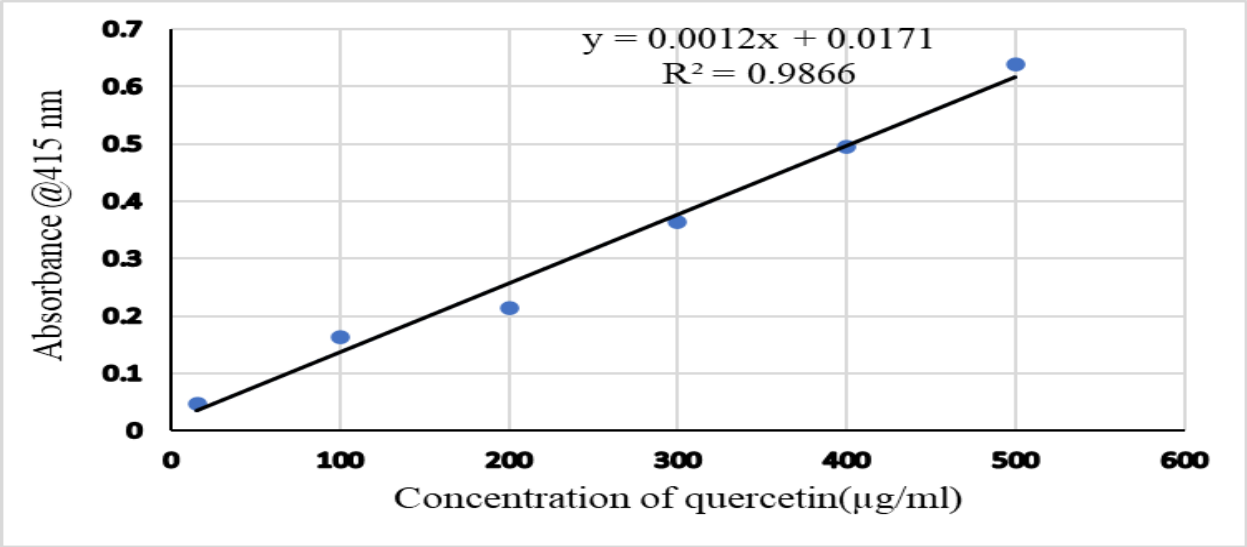

Figure 5: Quercetin standard absorbance graph.

As shown on the graph above the standard absorbance graph for Quercetin was expressed in a linear line with the equation Y= 0.0012X + 0.0171 and a correlation coefficient (R^2) of 0.9866. also, the absorbance for the sample resulted in 0.361 and Total Flavonoid Content (TAC) 286.58 mg QC/g dry extracts was calculated.

5. Discussion

This study was conducted to asses both *in vitro* and *in vivo* antidiabetic activity of 70% ethanolic extract of *R.abyssinica*. The experimental plant, *Rosa abyssinica*, is the only *rose* native to Africa which belongs to the big Rosaceae family [121]. This specific family of plants is very significant for management of diabetes mellitus, especially in underdeveloped nations with limited resources [122]. In this genus of plants, *Rosa*, are recognized to have cardioprotective, neuroprotective antihyperglycemic, anti-inflammatory and anti-constipation properties [123]. Additionally, studies on phytochemicals have reveled *Rosa* genus possessed with broad variety of secondary metabolites [123].Concidering how traditionally peoples use the plant, macerating with a traditional alcoholic drink called "Araki", for medicinal purpose specially diabetes and to get the maximum extraction power, the solvent 70% of ethanol was used for crude extraction process [88].

Considering variability exhibited by diabetic mellitus and different pathways a new agent might have pharmacological effect, it was advantageous to employ more than one animal model and both *in vivo* and *in vitro* models were utilized. Initially, *in vitro* α-amylase inhibitory activity was carried out in this investigation to determine the antihyperglycemic properties of 70 % ethanol fruit extract of *Rosa abyssinica.*

Reducing postprandial hyperglycemia is one of the best ways to manage diabetes. This is done by blocking the small intestinal brush border's enzymes that hydrolyze carbohydrates, alpha-glucosidase and alpha-amylase, which break down oligosaccharides and disaccharides into monosaccharides so that can be absorbed [124]. Acarbose, a well know medication that is frequently used to treat DM clinically, a pseudotetrasaccharide, is an α-amylase competitive inhibitor and its mode of inhibition appears to be because of the unsaturated cyclohexene ring and the glycosidic nitrogen linkage which resemble the transition state for the cleavage of enzymatic glycosidic linkages [124]. The list of compounds with human α-amylase inhibitory capacity includes flavonoids. The quantity of hydroxyl groups on the B ring of the polyphenol ligands flavonoid skeleton, which forms hydrogen bonds with the catalytic residues of the enzyme's binding site, determines how potently these substances block the enzyme [125]. Tannins also have α -amylase inhibitory activity because of their potent affinity for binding to proteins and carbohydrates to create indigestible and insoluble compounds that reduce the absorption of glucose [126]. The α-amylase inhibitory activity result demonstrates the plant's antidiabetic properties by reducing postprandial glucose absorption,

given that the IC50 values did not change significantly between the positive standard reference, acarbose, and *Rosa abyssinica's* extract (21.37 ± 4.25 μg/ml for acarbose and 26.72 ± 3.59 for *Rosa abyssinica*). The strong correlation between increasing concentration of extract/acarbose and the corresponding increment in α-amylase inhibition activity was indicated by higher R^2 value with all measurement above 0.9 which implies statistically significant concentration dependent α-amylase inhibition activity [120].The α-amylase inhibitory property of *Rosa abyssinica*'s extract maybe most probably due to its secondary metabolites i.e., flavonoids and tannins.

Beside the *in vitro* study, *in vivo* investigations were also carried out to ascertain the antidiabetic activity of *Rosa abyssinica* fruit extract. How the extract affects blood glucose level was performed in normal mice for hypoglycemic activity, in orally glucose loaded normal mice and stz- induced diabetic mice for antihyperglycemic activity.

The normoglycemic study revealed that except with the highest dose of extract, RA400, at 3 and 4 hrs with $P < 0.05$, the other doses of 70% ethanol fruit extract of *Rosa abyssinica*, RA100 and RA200 showed no hypoglycemic activity compared to the negative control group which indicates lower risk of hypoglycemia with use of this plant extract. However, the hypoglycemic effect resulted at 3 and 4hrs with regard to the highest dose of the extract, RA400, most likely indicates that the extract at its highest dose have similar activity like the positive control drug glibenclamide [127]. but the possible mechanisms of action need further confirmation. The slight reduction in BGL for the negative control group might due to extended fasting time.

The rise and fall in blood glucose levels after oral or intravenous glucose administration have long been used both clinically in patients and experimentally in animals as a measure of an antidiabetic agent's or plant extract's effectiveness in controlling blood glucose levels [128]. There are discrepancies among researches regarding sex preference to the sensitivity of glucose induced insulin secretion [105]. However, for both normoglycemic and oral glucose tolerance (antihyperglycemic activity) test male mice were chosen because female animals manifest improved glucose tolerance, likely due to greater insulin sensitivity in liver, muscles and adipose tissue [129] and also leptin signaling within the brain is sexually different to influence the regulation of glucose homeostasis and fat distribution, with females relying on leptin to a greater extent than males [130]. The mice were first fasted for 14hrs. (overnight fasting) before glucose loading. Fasting was required before glucose administration to provide stable baseline glucose measurements

because overnight fasting provokes a catabolic state in mice, as they primarily consume at night, depletes liver glycogen stores and eliminate fluctuations in BGL by food intake [131]. It also stimulates glucose induced insulin sensitivity [132]. 1.5g of glucose/kg body mass of mouse was given orally [106]. Even though both oral gavage (OG) or intraperitoneal (i.p.) injection routes of administration are generally accepted as appropriate, oral routes was chosen because plasma glucose levels are significantly lower in response to oral GTT compared with the same glucose dose administered i.p [133]. It primarily results from the release of gastrointestinal hormones Glucagon-like peptide (GLP) and glucose dependent insulinotropic polypeptide (GIP]), which significantly potentiate glucose-induced insulin release and result in lower blood glucose levels when compared with intraperitoneal administered glucose [134].

The effect of extract in lowering BGL in OGTT might point that the extract has capability of stimulating insulin release and the secreted insulin would then stimulate peripheral glucose uptake in muscle, fat and liver-tissue. In animal models of OGTT, secreted insulin requires greater than 2hrs to bring back the glucose level to normal [105]. In this experiment mice in the negative control group took 2hrs to bring the blood glucose level even closer to the normal level but the positive control and some higher dose of extract brought the BGL to the normal level with in much less time than the NC. The positive control group, GLC5, prevent the physiological induction of DM with in the first 30 minutes to 39.59% compared with NC of 107.4%. The highest dose of extract, RA400, and RA200 also showed significant reduction after the glucose challenge and started decreasing BGL at all time points. But the RA100 failed to show any significant reduction compared with the NC group. Since, the extract lowered BGL following glucose challenge these indicates that the extract might has insulin like action or it potentiates insulin action, insulin release from beta cells or facilitate peripheral absorption of the glucose to the muscle, fat or liver tissues.

In the single dose antidiabetic activity study of the extract, *Rosa abyssinica* showed remarkable antihyperglycemic effect. Especially the two higher doses of the extract, RA200 and RA400, brought the blood glucose level near to the normal range starting at 3 and 4 hrs. with highly significant ($p<0.001$) level. These two, RA200 and RA400, extract doses showed similar and comparable antihyperglycemic activity with regard to the positive control group, GLC5. The declines in FBG level in negative control group is attributed to extended fasting time but showed highly significant difference with the two higher doses of the extract. Even though the smaller dose

of the extract, RA100, did not show any significant difference but slight antihyperglycemic activity was noticed.

The primary goal of repeated dose antidiabetic activity study of the extract was to characterized the antidiabetic profile of the extract following repeated dose administration. This includes identification of potential target, hypothesizing mechanism of action and improvement in disease progress. The hyperglycemic effects of streptozotocin also last for a period of 8 weeks as indicated by in the negative control [105] so the repeated dose study was necessary to identify the effects of chronic administration of the extract and to see it's antidiabetic effect. The mice from the single dose study were continued to get the extract and controls daily for 3 weeks and their weekly blood glucose level was analyzed. Mice in the positive control group, GLC5, starting at week 2 and the two higher doses of the extract, RA200 and RA400, starting at week 3 managed to brought the weekly blood glucose level back to the normal control group level and showed significant difference with the negative control group. The smaller dose extract, RA100, however, failed to show any significant reduction. Moreover, the negative control showed increasing blood glucose level even more than the starting point. From the result, in addition to insulin like activity, that the extract has glibenclamide like activity indicates that 180 mg/kg STZ didn't destroy all beta cells, and the extract might activate the b cells to release insulin through SUR receptor activation.

Streptozotocin causes ulcers, diabetes, hepatotoxicity, nephrotoxicity, and severe weight loss [144]. A major anabolic hormone in the body, insulin, affects protein and fat metabolism as well as glucose metabolism when inadequate. The unopposed actions of the counter-regulatory hormones are also crucial in the development of metabolic disorders. When insulin levels drop, the balance shifts from the insulin-promoted anabolism of proteins and fats to catabolism. Following proteolysis, the liver removes gluconeogenic amino acids and uses them to produce glucose [145]. The weight loss may also result from polyureic nature of the mice after the diabetic was induced with STZ. The body of mice in the negative control group started showing significant week 1 ($p<0.01$), week 2 and 3 reduction ($p<0.001$) compared with the normal control group. But mice that took the GLC5, RA200 and RA400 showed highly significant different with the NC and showed almost comparably equal improvement like the normal control group but RA100 failed to show any improvement in body weight reduction. The extract may have potential as an antidiabetic due to its ability to enhance glycemic control and structural protein synthesis, as evidenced by the

smaller body weight loss in both the extract and standard medication treatment groups. This might be related to structural protein synthesis or an improvement in glycemic control [146].

The presence of secondary metabolites in medicinal plants, such as phenols, flavonoids, tannins, alkaloids, and saponins, contributes to their ability to restore the function of pancreatic tissues by increasing insulin output, inhibiting intestinal glucose absorption, or facilitating metabolites in processes that are insulin dependent. They also have free radical scavenging potential and antioxidant properties [105,147].The presence of most of these secondary metabolites including alkaloids (83.37 mg ATP/g), phenols (432 mg GAE/g) including flavonoids (286.58 mg QC/g) and others as described above at preliminary phytochemical screening contributed to the antidiabetic activity of the medicinal plant *Rosa abyssinica*.

6. Conclusion

This experimental study confirmed that the antidiabetic activity of 70% fruit extract of *Rosa abyssinica* with tolerable acute oral toxicity and positive result for all tested phytochemical secondary metabolites. The results obtained from the *in vitro* studies suggested that the extract works by reducing post-prandial hyperglycemia through α-amylase inhibition activity. The *in vivo* study, on the other hand, showed that oral administration of the extract of *Rosa abyssinica* has a beneficial effect in reducing blood glucose level both in oral glucose loaded and streptozotocin-induced diabetic mice, with a minimum risk of hypoglycemia.

7. Recommendation

The usage of medicinal plants for treatment of chronic diseases like diabetes and other is traditionally rooted in developing countries and still an essential part of public healthcare systems. The antidiabetic effects of *Rosa abyssinica* seen both *in vitro* with the ability to reduce postprandial hyperglycemia and *in vivo* with antihyperglyimic activity with less hypoglycemic side effect calls upon further advanced study on the plant to identify the exact mechanism of action and specific compound that result this activity. Antioxidant activity of the plant and streptozotocin's histopathological effect that occurred in the major organs of the diabetic mice (i.e., liver, pancreas, kidney) and the effect resulted from the repeated dose study of the plant need to be evaluated.

8. Reference

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