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Review Article

Antidiabetic Effects of Hydroethanolic Rhizome Extract of Anchomanes Difformis (Araceae) in Streptozotocin-Induced Diabetic Rats

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Abstract

Great efforts are ungoing in understanding and management of diabetes, the disease and disease related complications are increasingly unabated. In spite of the presence of known antidiabetic medicine in pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. A. Difformis (Araceae) is a self- growing herbaceous plant that grows in the tropical forest and is very frequently cited as a medicinal plant used in the treatment of cough, diarrhea and diabetes in southwestern Nigeria. The aim of this study is to evaluate the hypoglycaemic effect of the hydro-ethanolic extract of the rhizome of A. Difformis.

The effect of A. Difformis (125-500 mg/kg) on blood glucose levels in normal and glucose loaded rats and STZ induced diabetic rats were determined. After 21days of sub-acute treatment, blood samples were collected from rats for lipid, biochemical and insulin profiling.

In normal rats, A. Difformis did not cause significant reduction in blood glucose. At a dose of 500mg/kg, a significant decrease in blood glucose levels was observed 60 min after glucose load. A. Difformis at 500 mg/kg administered acutely showed a signicant ($p < 0.0001$) decrease in blood glucose level in diabetic rats. Administered sub acutely, the extract at 500 mg/kg significantly ($p < 0.0001$) reduced glucose levels at day 7 with subsequent decrease at day 14 and 21.The extract did not show any significant reduction in fasting blood insulin levels and biochemical parameters when evaluated showed no significant change when compared to control.

The hypoglycaemic effects of A. difformis is by reduction of blood glucose levels through possibly combination of mechanisms. This pharmacological evaluation validate the claims and its use as an anti-diabetic medicinal plant in Nigeria.

Keywords: Antidiabetic; Hydroethanolic; Rhizome; Anchomanes Difformis; Streptozotocin

Introduction

Plants are used as valuable sources of food and medicine for prevention of illness and maintenance of the health of human. In Nigeria, especially among the Hausa, indigenous plants are usually consumed as food or remedies in the treatment of human and animal diseases. The importance of medicinal plants has grown exponentially now that the World is turning to plants sources for drugs, supplement sand herbal preparation for the management of human health. Moreover, due to consistent scientific verification of safety and efficacy, herbal products are more acceptable than ever before, and therefore becoming economy booster [25]. The continual usage of medicinal plants as therapeutic agents for treatment of diseases in traditional medicinal system has brought about the need to carry out necessary test to ascertain safety and efficacy of folkloric preparations that are yet to be evaluated. Since creation, man has depended on plants for food, drinks, shelter, equipment, dental care and medicine [23].

Diabetes is a metabolic disease which exists everywhere in the world and affects approximately 6% of the world population. Nigeria has 24 to 33% of diabetics [18]. This prevalence rate places diabetes as one of the most frequent of endocrine diseases [24]. This disorder is caused by both genetic and exogenic factors (viral, chemical) leading to damage of the β cells of Langerhans, in the pancreas [30]. As a result of this, the body becomes unable to produce insulin, a pancreatic hypoglycaemic hormone. This disorder is characterized by polyuria (frequent and abundant urines), glycosuria (presence of glucose in urines) and hyperglycaemia (glucose rate on an empty stomach higher than 1.2g/l in plasma blood and confirmed in at least two occasions). Diabetes is a major cause of disability and death [17]. Currently, diabetes therapy is based on the use of hypoglycaemics (sulfonamides, biguanides, insulin), on hygieno-diet measures and exercises [47]. But the reasons for the continuous research for newer therapies through medicinal plants are limitless some include toxicity of these conventional drugs, cost of purchase, tolerance effects, pharmacokinetic properties just to mention a few.

In the search for means of fighting against this metabolic disorder, ethnomedicinal investigations were conducted in Africa and in most of the developing countries [8]. Diabetes seems to be an alarming disease and this has justified the need for finding newer affordable therapies, leading to the normalization of glucose levels and providing scientific evidence in the effectiveness of the use of traditional plants possessing anti-diabetic properties.

Anchomanes difformis is a self-growing herbaceous plant used in South -western Nigeria to treat diabetes. Could this ethinomedicinal claims be justified and proven pharmacologically?

Objectives

- To determine the anti-hyperglycemic effect of *Anchomanes difformis* following a normal glucose oral glucose tolerance test
- To determine the acute anti diabetic effect of *Anchomanes difformis* on STZ induced hyperglycemia
- To determine the sub-acute anti diabetic effect of *Anchomanes difformis* on STZ induced hyperglycemia.

Literature Review

Anchomanes Difformis

- Kingdom: Plantae
- Phylum: Streptophyta
- Class: Lilopsida
- Order: Alismatales
- Family: Araceae
- Subfamily: Aroideae
- Genus: Anchomanes
- Species: A. difformis
- Botanical name; *Anchomanes difformis*

Common name

Forest Anchomanes (Morton)

Vernacular (local) name

- Lángbòdó (Yoruba, SW Nigeria)
- Olumahi (Igbo,SE Nigeria)
- Hantsar Gadaa (Hausa, N Nigeria)

Description

Anchomanes difformis is a self-supporting rhizomatous herb with stout prickly stem (2 m high) with huge, divided stems. The stem is green in colour with a diffused white colour at the base of the soil level that arises from a horizontal tuber of about 80 cm by 20 cm long [12].

Geographical distribution

The plant grows in tropical forests in moist shady places, occurring in the forest of Sierra Leone to West Cameroons, Kenya, Kigoma region of western Tanzania, much of tropical Africa from Liberia to Tanzania, south to Angola and Zambia.

Edible uses

The rhizome is eaten in time of food scarcity but only after special preparation [59]. Treatment requires prolonged washing and cooking. The Fula add hearth-ashes to the cooking water and then leave the root in water to macerate and ferment for several days. It can then be sundried and stored for later consumption. [12].

ETHNO medicinal uses

The rhizome has medicinal uses. In Guinea the rhizomes are used to make rubefacients and vesicants for external application, and alternatives for internal medication, but care has to be exercised on account of the caustic nature of the sap. In Ivory Coast the plant is considered to be a powerful purgative and is used to treat oedemas, difficult child-birth, jaundice, kidney pain as a poison antidote, and strong diuretic for treating urethral discharge, jaundice and kidney-pains: for these the root or the leaves and stems may be used [34]. The root pulped with potter's clay is applied to maturate abscesses [34]. The rhizome is considered in Gabon to be lactogenic [59] while in Casamance (Senegal) the leaves are used for this. Sap from the stem is used in Ghana as an eye-medicine and the liquid obtained after cooking the crushed leaves with other drug-plants is drunk in Ivory Coast as a cough-cure.

Folkloric uses

Apart from its edible functions, the plant is used in various superstitious practices. The Hausa consider the fruiting spadix with red berries in the preparation of love-charms. The Yoruba invoke the red berries in an incantation for protection against ṣọpọnna (smallpox) under the name of ògìriçakó, ògìrì being a Yoruba food or flavouring made from the fermented kernels of *Citrullus colocynthis* (Linn.) Schrad (Cucurbitaceae). The Ijo of the Niger Delta uses the corm for the appeasement of the dead [1].

Chemical constituents

Rhizomes of the plant from Ivory Coast have been reported to contain: carbohydrates 77%; proteins 12%; fats 0·6%; minerals 9% and a quantity of amino-acids. A strong presence of alkaloids is found in Nigerian specie. Used for irrigant purposes due to its high tannins and saponin contents.

Scientific studies

- The methanolic extract has been reported to posses analgesic, antipyretic and local anaesthetic activity [8]
- The methanolic extract has been reported to posses in-vitro anti-plasmodial activity [25]
- Chemical and toxicological study has also been carried out on Anchomanes difformis (England) [57].
- The pharmacological constituents of the methanolic extract of the rhizome of Anchomanes difformis have also been investigated for its antimicrobial action [10].
- In vitro trypanocidal effect of methanolic extract of Anchomanes difformis [6].
- The toxic properties of forest Anchomanes, Anchomanes difformis against pulse beetle Callosobruchus maculates (Coleoptera: Bruchidae) [3].
- Comparative studies of the phytochemical and antimicrobial properties of the leaf, stem and tuber of *Anchomanes difformis* [43].
- The Ethyl Acetate Fraction of *Anchomanes difformis* has been to reported to posses Gastroprotective properties (England) [44].
- Anti-nociceptive and anti-inflammatory properties of the extract of *Anchomanes difformis* in albino rats [11].

Diabetes mellitus (DM)

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action [55].

Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome [54].

Figure 1: The leaf of *Anchomanes dwifformis.*

Figure 2: The Rhizome of *Anchomanes difformis.*

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [4]. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories (discussed in greater detail below). In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter

category, a degree of hyperglycemia sufficient cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected [5]. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load.

The degree of hyperglycemia (if any) may change over time, depending on the extent of the underlying disease process. A disease process may be present but may not have progressed far enough to cause hyperglycemia. The same disease process can cause impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) without fulfilling the criteria for the diagnosis of diabetes. In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose lowering agents. These individuals therefore do not require insulin. Other individuals who have some residual insulin secretion but require exogenous insulin for adequate glycemic control can survive without it. Individuals with extensive β cell destruction and therefore no residual insulin secretion require insulin for survival. The severity of the metabolic abnormality can progress, regress, or stay the same [44]. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic process and its treatment more than the nature of the process itself.

Types of diabetes mellitus

Type 1 DM

This form of diabetes, which accounts for only 5-10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamate decarboxylase (GAD65), and auto antibodies to the tyrosine phosphatases islet antigen (IA-2). One and usually more of these autoantibodies are present in 85-90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong human leukocyte antigen (HLA) associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective. In this form of diabetes, the rate of β cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or keto acidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors which are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anaemia.

Idiopathic diabetes- Some forms of type1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go.

Type 2

This form of diabetes, which accounts for 90-95% of those with diabetes, previously referred to as non-insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not

have any of the other causes of diabetes listed above or below. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications [35].

Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance.

Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined [26].

Causes

The lack of insulin is the particular disorder in type 1 diabetes. The problem of type 2 diabetes is not lack of insulin production; Most of these patients produce variable, even normal or high amounts of insulin.

Syndrome X is the name given to a collection of metabolic conditions, highlighted by insulin resistance, but also reduces obesity (especially in the abdomen), high cholesterol, low HDL, cholesterol, high triglycerides, high normal blood sugar and hypertension. These symptoms are often called pre diabetic symptoms. Insulin resistance is one of the key features and dangers of this syndrome. Insulin resistance is a condition where the pancreas is able to manufacture more than enough insulin, but the cells have become resistant to insulin effects, this increase blood glucose as well as stimulates insulin induced metabolic functions (conversion of carbohydrates to fats leading to obesity). The constant intake of refined carbohydrates, leading to dramatic spikes in blood glucose followed by dramatic spikes in insulin secretions, is the primary cause of insulin resistance and then type 2 diabetes. It is for this reason that diet is possibly the most important aspect in treatment of type 2 diabetes and its precursor condition, syndrome X [35].

The first stage in type 2 diabetes is the condition called insulin resistance. Insulin helps the glucose to enter the cells, where it is used for energy. Most patients with type 2 diabetes have insulin resistance, which has both genetic and environmental influences. In patients with insulin resistance although insulin can attach normally to receptors on the liver and muscle cells, certain mechanisms prevent insulin from moving glucose into these cells where it is utilized. As a result body starts making more and more insulin and in the beginning, this amount is usually sufficient to overcome such as resistance, but during the later phases of the disease the insulin resistance but during the later phase of the disease the insulin resistance increase in severity and, blood glucose increase but at the same time the body is unable to use it properly and the body cells are actually starving for energy. Even with increased amounts of insulin demands of the body is not met because of increasing tissue resistance. Because the body does not use the insulin properly, blood glucose levels rise above safe levels the initial effect at this stage may be an abnormal rise in blood glucose levels right after a meal (called post-prandal hyperglycemia). Insulin resistance is often present years before hyperglycemia develops and is the best indicator of which patients are at greater risk for diabetes (60). Type 2 diabetes differs from type 1 diabetes in that type 1 diabetes is always caused by inability to make enough insulin.

Type 2 diabetes tends to run in families. Though there are many available and effective treatments for type 2 diabetes, it may be extremely difficult to achieve the desired ultimate target namely, lifelong restorations of normal glucose control. An adequate response to insulin in peripheral tissues (e.g., fat muscles) leads to decreased peripheral glucose uptake (predominantly in muscles) [51], which causes circulating blood glucose concentrations to rise. Hypergly-

cemia, in turn stimulates the pancreas to insulin secretions resulting in hyperinsulinemia. Early in the disease course but before the development of type 2 diabetes, the pancreas is able to overcome insulin resistance and maintain a glucose levels within the normal range. Later in the progression to impaired glucose tolerance and diabetes, the pancreas is no longer able to provide enough insulin to overcome the body's resistance and hyperglycemia ensues.

Elevation of blood glucose level is first observed in the post prandial setting, when significant glucose excursions occur. The result from a failure of "first- phase" insulin secretion, the earliest islet cell defect demonstrated in patients with type 2 diabetes [11]. As the diseases, manifested by further islet cell dysfunction, blood glucose elevations also occur in the fasting state.

In some patients with type 2 diabetes, a greater degree of pancreatic failure is the primary cause of hyperglycemia. Insulin resistance while usually present in these patients may play a smaller role. Such patients do not have the classic coexisting features of obesity and hypertension and infact may be lean. Some may demonstrate islet autoimmunity and can be considered to have late- onset, slowly progressive type 1 diabetes.

The other critical pathophysiological factor leading to hyperglycemia in type 2 diabetes is hepatic overproduction of glucose [15]. Both glycogenesis and gluconeogenesis are under the inhibitory control of insulin. Insulin resistance in the liver is characterized by relative failure of this inhibition. This process is thought to have a major contribution to fasting hyperglycemia in patients with type 2 diabetes.

Risk factor of type 2 diabetes

- Close family member like parents or sibling with diabetes
- Obesity
- Age more than 45
- Hypertension
- History of developing high blood sugar during pregnancy.
- High blood cholesterol level
- High blood levels of triglycerides.

Diabetic complications

Hyperglycemia (high blood glucose) causes complications in patients with diabetes, regardless of an acute nature (ketoacidiosis due to low insulin or hypoglycaemic shock due to insulin overdoses), most complications are as a result of years of unregulated and high serum glucose. These complications include increased heart rate disease, retinopathy, neuropathy ending in renal failure, nephropathy, foot and leg ulcers, impotence and the inhibition of many important metabolic enzymes. Most of these complications are due to hyperglycaemic induced increase reactive oxygen species (ROS) that causes glucose induced activation of protein kinase C, increased formation of glucose derived advanced glycation end products (AGEs) and increased glucose influx through the aldose reductase pathway [41]. These vascular complications are cumulative, but preventable. Maintaining proper blood glucose is vital to prevent these complications. Additionally, there are a number of natural ingredients that can prevent and even reverse the progress toward these devastating complications.

Symptoms of diabetes

- Frequent urination because of large volume of urine (Polyuria)
- Excessive thirst (Polydipsia)
- Hunger and eating more (Polyphagia)
- Loss of weight despite the large eating
- Less common symptoms include
- Tiredness
- Headache and pain
- Blurring of vision
- Dry skin
- Dry mouth
- Impotence (in male) and vaginal yeast infection (in female)
- Difficulty in healing of wounds and scrapes.

Diagnosis of diabetes mellitus

Simple diagnosis can be done through the observation of the common symptoms and signs.

Symptoms of type 1 diabetes mellitus

- Increased thirst
- Increased urination
- Weight loss in spite of increased appetite
- Fatigue
- Vomiting

In type 1 diabetes, there is a very high concentration of ketone in the blood which have a fruity characteristic odour or smell of nail polish remover, which could be perceived from the patient's breath.

Symptoms of type 2 diabetes mellitus

- Increased thirst
- Increased urination
- Increased appetite
- Blurred vision
- Fatigue
- Impotence in men
- Slow healing infection

Signs and tests

Blood and urine tests

A urine analysis may be used to look for glucose and ketone from the breakdown of fat. However, a urine test alone does not diagnose diabetes. The following blood glucose tests are used to diagnose diabetes

- **Fasting blood glucose:** Diabetes is diagnosed if blood glucose level is higher than 126 mg/dL on two occasions. Levels between 100 and 126 mg/dl are referred to as impaired fasting blood glucose or prediabetes. These levels are considered to be risk factors for type 2 diabetes and its complications.
- **Random (non-fasting) blood glucose level:** Diabetes is suspected if blood glucose level is higher than 200 mg/dL and accompanied by the classic symptoms of increased thirst, urinations and fatigue.
- **Oral glucose tolerance test:** Diabetes is diagnosed if the blood glucose level is higher than 200 mg/dL after two (2) hours. This test is used more for diagnosis type 2 diabetes mellitus.

Patients with type 1 diabetes usually develop symptoms over a short period of time, and the condition is often diagnosed in an emergency setting. In addition to having high blood glucose levels, acutely ill type 1 diabetics have high blood levels of ketones. Ketones are produced by the breakdown of fat and muscles, and they are toxic at high level. Ketones in the blood cause a condition called acidosis (low blood pH).Urine testing detects both glucose and ketones in the urine. Blood glucose levels are also high.

Treatment goals

Current American Diabetes Association (ADA) goals for the treatment of diabetes are as follows

- The first goal in therapy is to reach and maintain an optimal fasting blood glucose level. This can be done in a way that benefits other metabolic outcomes such as improving lipid profile and reducing blood pressure.
- The second goal in therapy is to prevent and treat chronic consequences and complications associated with years of poor blood sugar control.
    - **Pre-prandial blood glucose level:** 80 to 120mg/dL (4.4 to 6.7mmol/L).
    - **Bed time blood glucose level:** 100 to 140mg/dL (5.6 to 7.8mmol/L)

The benefits of high blood glucose control were clearly showed for patients with type 1 diabetes. The importance of intensive control in type2 diabetes was confirmed by the kumamoto study [50]. Both trials demonstrated that when glucose concentrations are lowered towards the normal range, micro vascular complications associated with type 2 diabetes are reduced.

Achieving the easier in recent years with the advent of several new classes of oral medications. However, the presence of these

classes on the market, with their unique mechanisms of action and their ability to be used in complications, has made type 2 diabetes more complex to manage.

Treatment options

Diet and lifestyle

It is difficult to over emphasize the importance of diet as it pertains to preventing and treating type 2 as well type 1 diabetes. Since diabetes is a metabolic disorder, food can be considered either a poison or a therapy depending on its contents. Not only can food lead to obesity, but food is also the primary foundation for blood sugar control often called glycemic control or balance. The steady balance of glycemic control is the preventing and treating syndrome X and type 2 diabetes. Avoiding food that destabilize glycemic balance is the key to a healthy diet for everyone, but especially for those with insulin resistance. Other lifestyle factors are also important for those with diabetes or syndrome X. Exercise and stress management are vital. Physical activity and regular exercise may not only have direct impact on glucose and insulin sensitivity, but also on many risk factors such a obesity, triglycerides and hypertension. In a recent study, exercise had significant improvement on vascular function in type 2 diabetes [38].

Stress management

Along with the pancreatic production of insulin, the adrenal glands are important in the regulation of blood sugar. The adrenal hormone cortisol is stimulated whenever the body is "under stress". Whether this stress comes from mental or emotional stress, chronic inflammation, food allergies, low blood sugar, and cortisol effectively raised blood glucose level by stimulating gluconeogenesis. This raised glucose level can exceed desired levels when stress-induced cortisol levels are extremely high (alarm reaction).Chronic glycemic imbalance or other stress could then result in both reduced sensitivity and adrenal exhaustion. Studies have showed that glycemic control of diabetes with higher measurable stress is worse than these with lower measurable stress and additional techniques to reduce stress lead to better glycemic control among diabetic patients [58]. Reports have also directly linked the function of the hypothalamic- pituitary-adrenal axis with the risk of type 2 diabetes [48]. An excellent way to determine adrenal stress is by measuring salivary cortisol throughout a single day.

Medications for type 2 diabetes

Five classes of oral anti-diabetic agents have been approved by the US food and administration. Certain properties may be specific agents more or less appropriate for a specific type of patients.

Oral agents in the treatments of type 2 diabetes

- Medications that increase the insulin output by the pancreas
    - Sulfonylurea
    - Meglitinides
- Medications that decrease the amount of glucose produced by the liver
    - Metformin
- Medications that increase sensitivity of the cells to insulin
    - Thiazolidines
- Medications that decrease the absorption of carbohydrates from the intestine
    - Alpha-Glucosidase inhibitors

Medication that increases the insulin output by the pancreas

Sulfonylurea

Despite the development of several new classes of drugs, sulfonylurea still plays a primary role in the treatment of type 2 diabetes. In the early 1950s, sulfonylurea deputed as the first class of oral anti-diabetic agents. Sulfonylurea promotes increased pancreatic insulin secretion through interaction with sulfonylurea receptor on the islet cells. They target pancreatic secretory dysfunction and raise serum insulin levels high enough to overcome insulin resistance in peripheral tissues. Increased insulin flow directly into the portal vein, decreasing hepatic glucose production [52]. These drugs are effective in rapidly lowering blood sugars but run the risk of causing hyperglycemia. In addition, they are sulfa compounds, and should be avoided in patients with sulfa allergies.

- **First generation sulfonylureas**
    - Generic Trade name
    - Tolbutamide Orinase
    - Tolazamide Tolinase

- Chlorpropramide Diabinase

- **Second generation sulfonylurea**
  - Generic Trade name
  - Glipizide Glucotrol
  - Glyburide Micronase, Diabeta
  - Micronized gly Glynase

- **Third generation sulfonylurea**
  - Generic Trade name
  - Glimepiride Amaryl

Meglitinidines

This drug has a mechanism of action similar to sulfonylurea and the side effects also resemble that of sulfonylurea. Repaglinide is a suitable option for patients with severe sulfa allergy who are not candidates for sulfonylurea therapy. The drug is used alone or in combination with metformin.

Medications that decrease the amount of glucose produced by the liver

Biguanides

Metformin, a biguanides, was introduced into the United States in 1995. The drug probably leads to some improvement in peripheral insulin sensitivity but appears to exert most of its effects on the liver [29]. Recently, it has been shown that the decrease in hepatic glucose production with the metformin therapy is due to reduction in gluconeogenesis [28]. The risk of lactic acidosis with metformin is markedly reduced from that with its predecessor, phenformin, which is removed from the market in 1977. The incidence of lactic acidosis from the metformin is only 0.03 per 1,000 patient-years of use. Studies have consistently shown that metformin also lowers fasting blood glucose levels by about 60 to 70 mg/dL (3.3 to 3.9 mmol/L). It may, therefore, be an appropriate first line therapy for patients of any weight. Metformin is used alone or in combination with sulfonylureas for the treatment of type 2 diabetes.

Medications that increase the sensitivity of the cells to insulin

Thiazolidinediones

These groups of drugs are called "insulin sensitizers" that promotes uptake of glucose by the muscle. Troglitazone is the first agent of this drug from this class was introduced for the treatment of type 2 diabetes. This drug should not be used alone. Gthos drug was withdrawn from the market due to its side effects. Rosiglitazone (Avandia®) and pioglitazone are the newer drug in this group.

Medications that decrease the absorbtion of carbohydrates from the intestine

Alpha-glycosidase inhibitors

Acarbose (precise®) and Miglitol (glyset®) are the commonly used drug in this group and can be used alone or in combination with sulfonylurea. These drugs block the breakdown of complex carbohydrates and delay the absorption of glucose from the gastrointestinal tract. The dose should be increased slowly to decrease the side effects of flatulence and gastrointestinal upset.

Insulin

Even though type 2 diabetes patients are generally resistance to the action of insulin, this drug may be a reasonable choice in some situations. It is a peptide hormone produced by the beta cells in the pancreas, composed of 51 amino acids, and has a molecular weight of 5808g. It is a dimmer of an A-chain and a B- chain, which are linked by a disulfide bond. Insulin therapy can prevent diabetes complications by helping keep your blood sugar within target range [26].

Induction of diabetes

In the 1880s, Von Mering was working on the absorption of fat from the intestine when Minkowski suggested he remove the pancreas of a dog. The animal developed polyuria and polydipsia and was found to have diabetes mellitus. Many experiments on rabbits and dogs followed, although history has given a special place to Marjorie, one of the dogs used by Banting and Best in their seminal experiments on the isolation and purification of insulin in the 1920. Marjorie is probably the most famous experimental animal in history, only to be superseded by Dolly the Sheep in recent years.

One of the most straight forward ways of studying the effects of hyperglycaemia in an animal is to remove the pancreas, either partially or totally. The species of animal used is determined by several factors. In general, the smaller the animal, the more manageable and cheaper the experiment. Hence, pancreatectomised rats and mice are the most commonly used. One of the guiding principles of animal research is to use the 'lowest' possible animal and, nowadays, permission would not be granted to remove the pancreas of a dog unless a similar experiment could not be performed in a rodent. However, a major criticism of using rodents is that they may not adequately reflect the human situation and occasionally justification is sought to use larger animals such as cats, dogs, pigs and primates. Non-surgical methods of inducing hyperglycaemia by damaging the pancreas also exist. These include the administration of toxins such as streptozotocin [19] and alloxan [14].

Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) was first described by Brugnatelli in 1818. Wöhler and Liebig used the name "alloxan" and described its synthesis by uric acid. Alloxan exerts its diabetogenic action when administered parenterally: subcutaneously, intravenously or intraperitionally the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse [20]. The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg b. w. [27]. When alloxan is given intraperitonealy or subcutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg b. w. may be insufficient for inducing diabetes in the rat [33] Fasted animals are more susceptible to alloxan [33,56], whereas increased blood glucose provides partial protection [7,56].

The mechanism of alloxan action has been predominantly studied. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells.

Streptozotocin

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3nitrosoureido)-D-glucopyranose) is synthesized from *Streptomycetes achromogenes* and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively). The range of the STZ dose is not as narrow as in the case of alloxan. The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b. w. [21], but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg b. w. may be ineffective [33]. Streptozotocin enters the B cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, B cells undergo the destruction by necrosis.

Chapter three
Materials and Method

Apparatus

Weighing balance (Mettler PM 480 Delta range Switzerland), syringes, oral cannula (Ysale 20, B.D.2D), Needles, Oven (Gallenhamp England), cages, Blades, Glucometer and glucometer strips (Acu check®, USA).

Drugs and reagents

Glibenclamide (Pomdanil®, May and Baker, Nigeria), Streptozotocin (Sigma Chemical Co., St. Louis, USA), Insulin (Humulin®, USA)

Experimental animals

Male albino rats (100g-150g) and mice (25-30g) used were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos. The animals were acclimatized and fed with standard animal feed (Livestock Feed Nigeria Plc.) and allowed to drink tap water *ad libitum*..

Plant source

Fresh plant of *Anchomanes difformis* consisting of the leaves, stem and rhizome were collected in June 2014 from a farm in Ikire, few kilometres from Ibadan, Oyo state and was identified and authenticated at the Forestry Reserve Institute of Nigeria (FRIN) Ibadan by Mr T. K. Odewo. The specimen identification number LUH 6110 was obtained from university of Lagos herbarium.

Extraction process

The rhizome of *Anchomanes difformis* was washed, chopped and dried in shade for about three weeks. The dried rhizomes were grinded into powder. The powder was then macerated in ethanol and water in the ratio 70:30 respectively, the mixture was stirred frequently for a period of 72 hours then filtered. The residue was re-macerated in ethanol and water to ensure exhaustive extraction and the filtrate was oven dried to get brown extract. Total yield of extract gotten was 10.7%; this was derived using the formula: weight of extract ÷weight of starting plant material × 100.

Weight of extract = 107g

Weight of starting plant material = 1000g

% Yield = 107 ÷ 1000 ×100 = 10.7%.

Physicochemical properties

The physicochemical properties (pH, smell, taste, texture and colour) of the hydro-ethanolic extract of *Anchomanes difformis* were examined.

Acute toxicity

Two (2) groups of 5 animals each were fasted overnight; the animals were then treated with distilled water and extract at 5000 mg/kg orally. After 2hrs of administration, the animals were observed for behavioural changes (writhing, grooming, rearing, increase/decrease locomotion, piloerection, sedation/aggressiveness, response to touch, defecation, convulsion etc. Mortality within 24hrs was also recorded and used to determine the LD 50. Animals were further observed for 14days to check for signs of delayed toxicity.

Determination of blood glucose level

Six (6) groups of 8 rats each were fasted overnight (16h). The blood samples from the rats were then obtained by gently nipping the tail with a lancelet and squeezing the tail to allow 2-3 drops of fresh venous blood which were dropped on glucometer strips properly inserted into the glucose monitoring meter (Glucometer, Accu-chek®) and values were read on the digital display screen of the meter.

Effects on normal and glucose-loaded rats (NG-OGTT)

After obtaining the fasting blood glucose of randomly allotted rats, the test drugs were then administered as follows

- **Group 1:** Distilled water (10ml/kg p. o)
- **Group 2:** Insulin (4IU/kg s. c)
- **Group 3:** Glibenclamide (2.5mg/kg p. o)
- **Group 4:** Hydro-ethanolic extract ANCD 125 mg/kg p. o
- **Group 5:** Hydro-ethanolic extract ANCD 250 mg/kg p. o
- **Group 6:** Hydro-ethanolic extract ANCD 500 mg/kg p. o

The glucose levels were determined at 30, 60, 90, 120, mins post treatment, animals were then loaded glucose 2g/kg and the blood glucose levels were further determined at, 150,180 240, 300,360, and 420 mins (Aslan., *et al*. 2010).

Induction of diabetes

The fasting blood glucose level was determined in 16h fasted rats. Diabetes was then induced by a single intraperitoneal injection of freshly dissolved streptozotocin (STZ) 60 mg/kg in 0.1M sodium citrate buffer (pH 4.5) [21]. STZ was diluted in freshly prepared Na-Citrate buffer immediately prior to injection to avoid degradation of the STZ. The glucose levels were recorded 72hrs after streptozotocin administration and rats with blood glucose concentrations ≥200 mg/dL were considered diabetic.

Acute antidiabetic effect

Five (5) groups of 8 diabetic rats were randomly allotted. Treatment was then carried out as follows:

- **Group 1:** Distilled water (10ml/kg p. o)
- **Group 2:** Insulin (4IU/kg s. c)
- **Group 3:** Hydro-ethanolic extract ANCD 125mg/kg p. o
- **Group 4:** Hydro-ethanolic extract ANCD 250mg/kg p. o
- **Group 5:** Hydro-ethanolic extract ANCD 500mg/kg p. o
- **Group 6:** Non-STZ treated control

The blood glucose level of each of the experimental animals was determined at 30, 60, 120, 240, and 360min post-treatment.

Sub-acute anti-diabetic effect

Five (5) groups of 8 diabetic rats were randomly allotted. Treatment was then carried out as follows:

- **Group 1:** Distilled water (10ml/kg p. o)
- **Group 2:** Insulin (4IU/kg s. c.)
- **Group 3:** Hydro-ethanolic extract ANCD (125mg/kg p. o)
- **Group 4:** Hydro-ethanolic extract ANCD (250mg/kg p. o)
- **Group 5:** Hydro-ethanolic extract ANCD (500mg/kg p. o)
- **Group 6:** Non-STZ treated control

Treatment was continued once daily for the next 20 days. On the evening of day 20, all rats are fasted for 16h, then on day21, blood is collected from each rat through the retro- orbital sinus and serum is generated for biochemical, antioxidant assay and insulin profiling.

Insulin profiling

On the 21st day of the experiments, blood samples were obtained through the retro orbital sinus. Blood samples were then centrifuged and serum generated and the fasting insulin levels obtained.

Haematological analysis

On the 21st day of the experiments, blood sample were then obtained from the animals through the retro orbital sinus animals were sacrificed by cervical dislocation. Blood samples obtained were centrifuged for 15 minutes to obtain clear serum which were kept at 20°c for biochemical assay of cholesterol, urea, LDL (Low density lipoprotein), HDL (High density lipoprotein), bilirubin, ALP (Alanine phosphatase), AST (Aspartate transaminase), ALT (Alanine transaminase), total protein, triglycerides, creatinine, and glucose using COBAS lipid analyzers.

Statistical analysis

Statistical analysis was done using One and Two-way Analysis of Variance (ANOVA) followed by Tukey's post hoc multiple comparison test using GraphPad Prism 6 (GraphPad Software, CA, USA). Results were considered significant at $P < 0.05$

Chapter 4
Results

Physicochemical properties

The rhizome of *Anchomanes difformis* has a crystalline powdery texture, dirty brown in colour, with a characteristic smell. It has sweet taste and is highly soluble in water but sparingly soluble in methanol with a pH of 6.3.

Properties	Observation
Colour	Dirty brown
Smell	Characteristic
Taste	Sweet
Texture	Powdery crystalline
Solubility in water	Yes
Solubility in methanol	Incomplete
Ph	6.3
Characteristic on exposure to air	Not observable

Table 1: Physiochemical properties of the hydroethanolic extract of the rhizome *Anchomanes difformis*.

Acute toxicity

The hydroethanolic extract of *Anchomanes difformis* did not produce any mortality when administered once orally up to 5 g/kg. No visible signs of delayed toxicity and mortality were observed when the animals were monitored for further 14days.

Effect of hydro-ethanolic extract of *Anchomanes difformis* on Normal Glucose-Oral Glucose Loaded Rats

Compared to glucose level at 0 min., there was significant ($P < 0.5$, $P < 0.01$, $P < 0.0001$) reduction in glucose level in the insulin treated group at 30, 60, 90, 120 mins. *A. Difformis* at increasing dose concentrations (125, 250 and 500mg/kg) showed no significant reduction in blood glucose level. Glibenclamide, showed a significant reduction in blood glucose levels at 90 mins, 120 mins and 150 mins. After glucose administration at 120 mins, there was significant increase in glucose levels at 180mins in all treated group with the exception of the insulin group. Compared to the glu-

cose level at 180 min (120 min post-glucose load), Glibenclamide showed a significant reduction in blood glucose levels at 240, 300, 360 and 420 mins The extract of *A. Difformis* (125, 250 and 500mg/kg) showed significant ($p < 0.0001$) reduction in blood glucose level at 300, 360and 420 mins time interval.

Acute Anti-diabetic effect of hydro-ethanolic extract of *Anchomanes difformis*

Compared to the baseline glucose levels at 0 mins, there was significant reduction in blood glucose levels in the insulin, *A. Difformis* 125mg/kg and 500mg/kg. In the insulin treated group, sig-

Treatment	Time Interval (Mins)										
	0	30	60	90	120	150	180	240	300	360	420
Control (10 mL/kg)	83.13 ± 2.52	80.25 ± 2.03	77.63 ± 3.69	71.75 ± 4.67	63.75 ± 6.62*	82.75 ± 3.31	143.75 ± 4.60****,	98.13 ± 8.94^{4}	77.63 ± 4.35,^{4}	69.88 ± 3.25^{4}	71.25 ± 3.33^{4}
Insulin (4IU/kg)	75.13 ± 4.01	41.63 ± 2.38*	24.00 ± 2.56***	20.25 ± 1.86****	20.13 ± 0.95****	36.75 ± 9.47**	64.88 ± 14.18	101.25 ± 14.82	45.75 ± 7.06	50.25 ± 5.77	54.00 ± 5.17
Glibenclamide (2mg/kg)	73.63 ± 3.88	67.50 ± 5.62^{d}	62.63 ± 5.37^{d}	46.13 ± 2.39$^{**, d}$	40.13 ± 2.64$^{***, b}$	48.38 ± 2.92**	102.13 ± 11.64**,	56.00 ± 4.20$^{4, b}$	37.75 ± 3.88^{****,4}	33.13 ± 4.24$^{****,4, a}$	31.38 ± 4.51$^{****,4, b}$
ANCD 125mg/kg	77.63 ± 3.17	79.13 ± 2.88^{d}	76.88 ± 2.85^{d}	72.00 ± 2.73$^{d, κ}$	69.50 ± 2.62$^{d, κ}$	76.25 ± 3.76$^{d, β}$	135.88 ± 5.17$^{****, d}$	96.50 ± 5.18**	77.38 ± 2.51$^{4, c, κ}$	65.63 ± 2.40$^{4, κ}$	51.00 ± 3.83****,$^{4, β}$
ANCD 250mg/kg	66.00 ± 5.46	70.00 ± 2.40^{d}	73.63 ± 3.32^{d}	67.75 ± 2.84$^{d, γ}$	63.13 ± 2.63$^{d, β}$	76.25 ± 3.76$^{d, β}$	133.38 ± 10.17$^{****, c}$	97.25 ± 6.96^{**,3}	66.25 ± 5.61$^{4, a, β}$	53.50 ± 2.67$^{4, α}$	44.88 ± 2.27^{*,4}
ANCD 500mg/kg	82.13 ± 2.70	80.00 ± 2.69^{d}	76.88 ± 2.89^{d}	67.75 ± 2.89$^{d, γ}$	69.00 ± 4.12$^{d, κ}$	73.88 ± 4.25$^{d, α}$	137.75 ± 7.47$^{****, d}$	84.00 ± 5.47^{4}	76.63 ± 4.44$^{4, c, κ}$	70.13 ± 4.75$^{4, κ}$	60.38 ± 2.03$^{**,4, κ}$

Table 2: Effect of hydro-ethanolic extract of *Anchomanes difformis* on Normal Glucose-Oral Glucose Loaded Rats.

Values are expressed as Mean ± S.E.M (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. 0 mins ; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$ vs. Insulin; $^{α}P < 0.05$, $^{β}P < 0.01$, $^{γ}P < 0.001$, $^{κ}P < 0.0001$ vs. Glibenclamide;$^{1}p < 0.05$, $^{2}p < 0.01$,$^{3}p < 0.001$, $^{4}p < 0.0001$ vs 180mins (One -way Analysis of variance (ANOVA) followed by and Tukey's multiple comparison test.

Treatment	0	Time (Mins)				
		30	60	120	240	360
Diabetic Control (10mL/kg)	341.00 ± 63.30	339.33 ± 66.69	312.67 ± 62.23	311.33 ± 62.33	275.83 ± 54.32	249.67 ± 49.67
Insulin (4IU/kg)	458.50 ± 41.82	342.33 ± 47.28	275.50 ± 40.44*	249.17 ± 38.05**	225.17 ± 32.33***	207.17 ± 31.93***
ANCD 125mg/kg	486.00 ± 33.94	466.17 ± 52.14	299.50 ± 26.22**	288.00 ± 17.64**	206.00 ±31.23****	240.50 ± 34.32****
ANCD 250mg/kg	430.67 ± 44.56	352.67 ± 39.72	325.00 ± 46.15	276.50 ± 40.70	273.67 ± 52.91	337.00 ± 85.63
ANCD 500mg/kg	433.00 ± 42.00	311.00 ± 40.70	223.33 ± 27.82**	175.00 ± 27.73$^{****, a}$	179.17± 28.58****	171.67 ± 28.38****
Non-Diabetic Group	85.67 ± 2.50	80.50 ± 1.28,^{b}	80.00 ± 4.25,^{c}	75.67 ± 3.09,^{c}	70.17 ± 3.67$^{**, b}$	71.50 ± 3.78$^{*, a}$

Table 3: Acute Anti-diabetic effect of hydro-ethanolic extract of *Anchomanes difformis.*

Values are expressed as Mean ± S.E.M (n = 6).). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. Glucose level at 0 mins $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$ vs Diabetic control (One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

nificant ($p < 0.05$, $p < 0.01$, $p < 0.001$) reduction in glucose level was observed at 60, 120, 240 and 360 mins. At the dose of 500mg/kg, significant ($p < 0.0001$) reduction in glucose levels was observed at 120, 240 and 360 mins time interval.

Sub-acute anti-diabetic effect of hydro-ethanolic extract of *Anchomanes difformis*

In comparison to the day 1 glucose level, there was no significant reduction ($P < 0.05$) in glucose level on days 7, 14, and 21 in the diabetic control group. Insulin produced significant reduction ($P < 0.0001$) in glucose level. *A. Difformis* dose of 125mg/kg also produced significant reductions ($P < 0.05$, $P < 0.001$) in glucose level on day 7, 14 and 21. At a dose of 500mg/kg on day 7, 14 and 21, a significant reduction ($P < 0.0001$) in glucose levels was recorded.

Treatment	Initial BGL before STZ Induction (mg/dL)	BGL After STZ Induction (mg/dL)			
		Day 1	Day 7	Day 14	Day 21
Diabetic Control (DW 10mL/kg)	82.67 ± 3.39	341.00 ± 63.30	313.83 ± 59.88	229.50 ± 49.86	149.40 ± 25.11
Insulin (4IU/kg)	61.50 ± 6.05	456.17 ± 40.83	121.33 ± 5.52$^{****, b}$	93.33 ± 2.36$^{****, b}$	69.00 ± 3.00****
ANCD 125mg/kg	72.50 ± 3.82	486.00 ± 33.93	408.83 ± 26.71*	267.33 ± 27.39**	181.20 ± 29.66**
ANCD 250mg/kg	67.33 ± 3.77	430.67 ± 44.56	274.33 ± 48.92*	185.33 ± 21.56**	155.50 ± 14.84****
ANCD 500mg/kg	72.17 ± 5.27	433.00 ± 42.00	192.67 ± 11.06$^{****, a}$	155.00±16.09****	125.60 ± 4.96****
Non-Diabetic Group	70.83 ± 3.11	85.67 ± 2.50$^{****, c}$	81.50 ± 1.77$^{****, c}$	76.33 ± 6.32**,	84.67 ± 2.08

Table 4: Sub-acute anti-diabetic effect of hydro-ethanolic extract of *Anchomanes difformis.*

Values are expressed as Mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. Day 1, $^{a}p < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.0001$ vs diabetic control. (One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Effect of hydro-ethanolic extract of *Anchomanes difformison* Fasting Blood Insulin Profile

The fasting blood insulin level represented in table 5 showed a no significant ($P > 0.05$) increase compared with the different groups administered with the standard, control and with extract of *Anchomanes difformis* at different doses.

Treatment	Fasting Blood Insulin [Insulin Value (0.7-9.0µIU/mL)]
Distilled Water(10mL/kg)	6.73 ± 1.13
ANCD 125mg/kg	20.73 ± 15.10
ANCD 250mg/kg	9.00 ± 5.70
ANCD 500mg/kg	9.07 ± 0.70
Non-Diabetic Group	6.57 ± 1.36

Table 5: Effect of hydro-ethanolic extract of *Anchomanes difformis*on Fasting Blood Insulin Profile.

Values are expressed as Mean ± S.E.M (n = 3). $P > 0.05$. One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

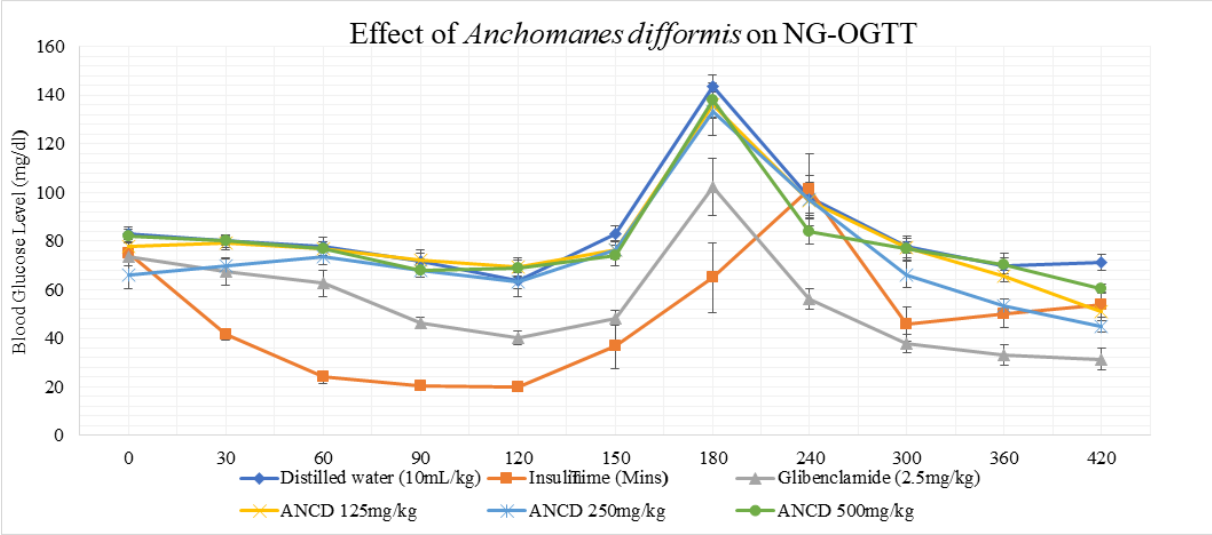
Effect of hydro-ethanolic extract of *Anchomanes difformis* on Haematological parameters of Blood serum

The extract at 125,250 and 500mg/kg showed no significant ($P > 0.05$) increase when compared with the standard drugs. There was an increase in the level of ALP as compared to control at doses of 125mg/kg and 500mg/kg rhizome *A. Difformis.*

Treatment	Haematological Parameters												
	UREA	GLU	CHOL	AST	BILT	CREA	ALT	ALB	S-TP	HDL	LDL	TRIG	ALP
Control (DW 10mL/kg)	6.04 ± 1.01	13.30 ± 3.21	0.48 ± 0.18	240.84 ± 43.60	1.20 ± 0.14	25.48 ± 1.48	52.30 ± 3.47	33.76 ± 1.42	60.40 ± 1.71	1.03 ± 0.11	0.30 ± 0.03	1.45 ± 0.10	282.70 ± 46.00
ANCD 125mg/kg	3.94 ± 1.33	15.36 ± 3.16	0.24 ± 0.06	191.34 ± 25.21	1.10 ± 0.13	23.67 ± 2.54	58.12 ± 5.95	28.48 ± 0.89	55.76 ± 1.74	0.81 ± 0.13	0.27 ± 0.04	1.18 ± 0.01	431.92 ± 34.23
ANCD 250mg/kg	6.05 ± 0.74	4.90 ± 0.36	0.51 ± 0.11	208.40 ± 25.60	1.23 ± 0.17	23.99 ± 0.57	68.08 ± 8.78	32.68 ± 3.43	60.95 ± 2.10	1.19 ± 0.20	0.34 ± 0.05	1.05 ± 0.17	376.35 ± 39.00
ANCD 500mg/kg	7.16 ± 1.13	13.742. 59	0.44 ± 0.10	219.50 ± 42.93	1.18 ± 0.27	22.54 ± 1.57	60.80 ± 6.97	28.90 ± 2.18	58.00 ± 3.87	0.83 ± 0.18	0.22 ± 0.03	1.81 ± 0.80	554.44 ± 53.21
Insulin (4IU/kg)	5.88 ± 0.12	4.00 ± 0.11	1.12 ± 0.05	176.40 ± 5.40	1.51 ± 0.10	19.64 ± 0.50	62.25 ± 0.95	26.65 ± 1.15	45.45 ± 1.15	1.31 ± 0.10	0.45 ± 0.02	2.02 ± 0.02	295.00 ± 16.00
Non-Diabetic Group	6.28 ± 1.34	6.73 ± 1.98	0.71 ± 0.11	188.33 ± 9.90	1.03 ± 0.23	18.71 ± 1.82	68.10 ± 8.15	30.40 ± 1.62	56.93 ± 2.06	1.29 ± 0.15	0.39 ± 0.07	1.29 ± 0.20	297.60 ± 38.75

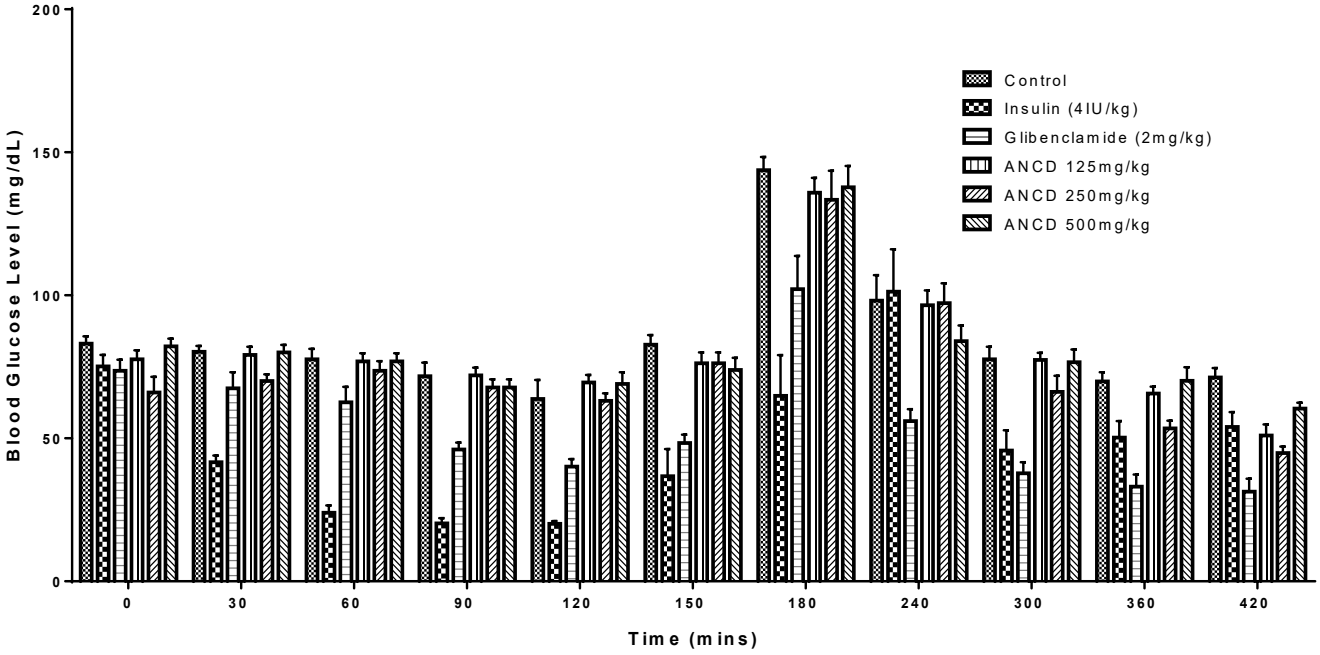
Table 6: Effect of hydro-ethanolic extract of *Anchomanes difformis* on biochemical parameters of Blood serum.
Values are expressed as Mean ± S.E.M (n = 6). P < 0.05 (One -way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Appendix 1: Line graph of normal glucose-oral glucose loaded tolerance test.
Line graph showing the effect of *Anchomanes difformis* on Normal glucose-oral loaded glucose tolerance test in rats. Values are expressed as Mean ± SEM (n = 8).



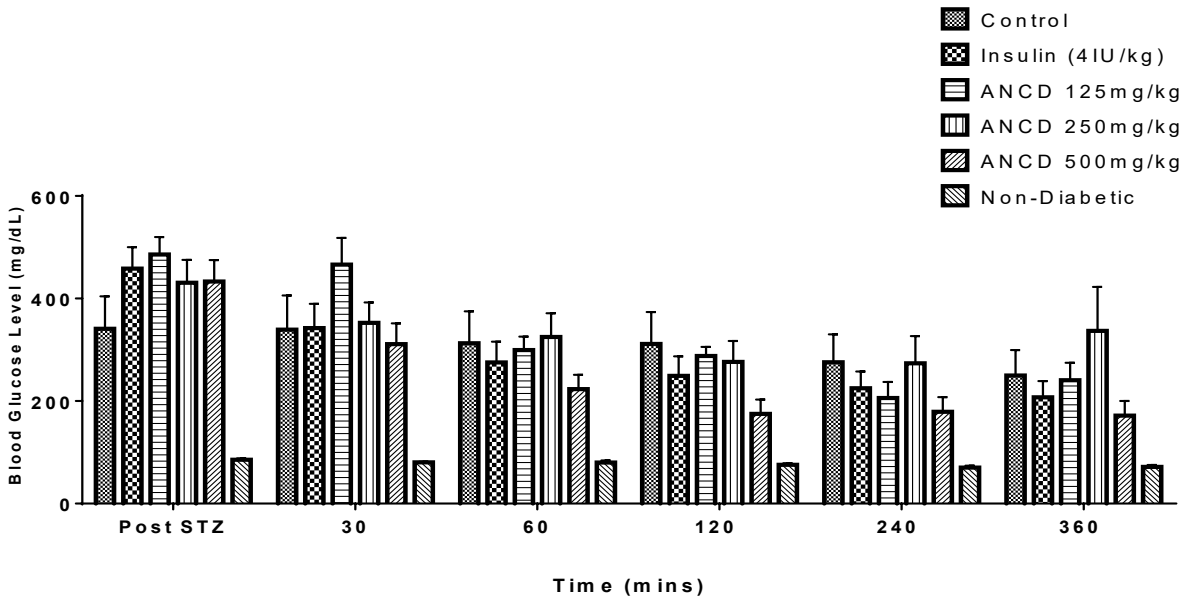
Appendix 2: Bar chart of Normal glucose-oral glucose loaded tolerance test.

Values are expressed as Mean ± S.E.M (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. Control; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$ vs. Insulin; $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$, $^{\gamma}P < 0.001$, $^{\kappa}P < 0.0001$ vs. Glibenclamide. (One -way Analysis of variance (ANOVA) followed by Dunnet's and Tukey's multiple comparison test.).



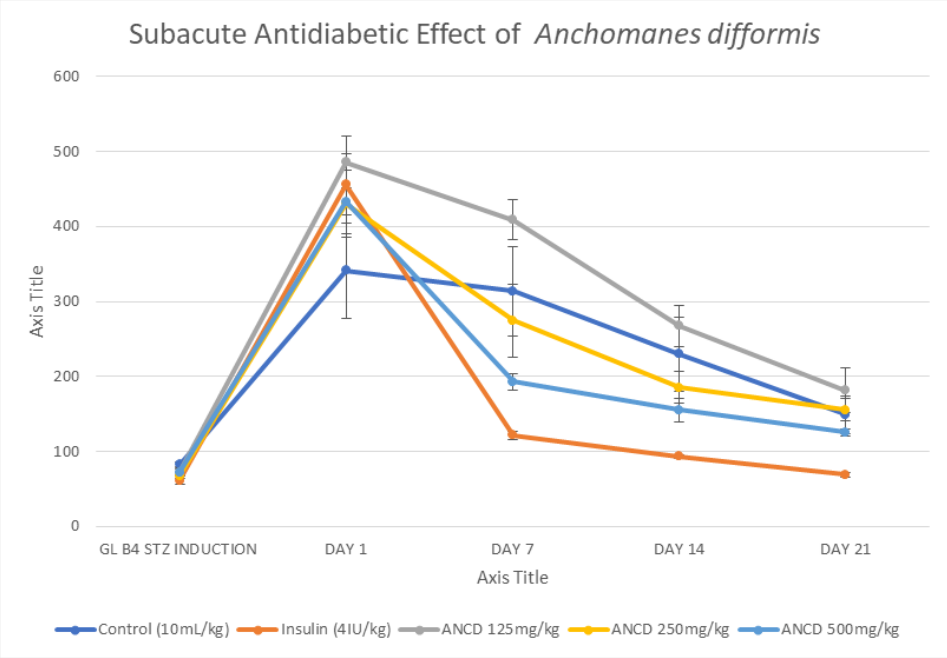
Appendix 3: Bar chart of acute anti-diabetic effect of *Anchomanes difformis.*

Values on bar chart are expressed as Mean ± S.E.M (n = 6). $^{*}P > 0.05$. (One way Analysis of variance (ANOVA) followed by Dunnet's and Tukey's multiple comparison test).



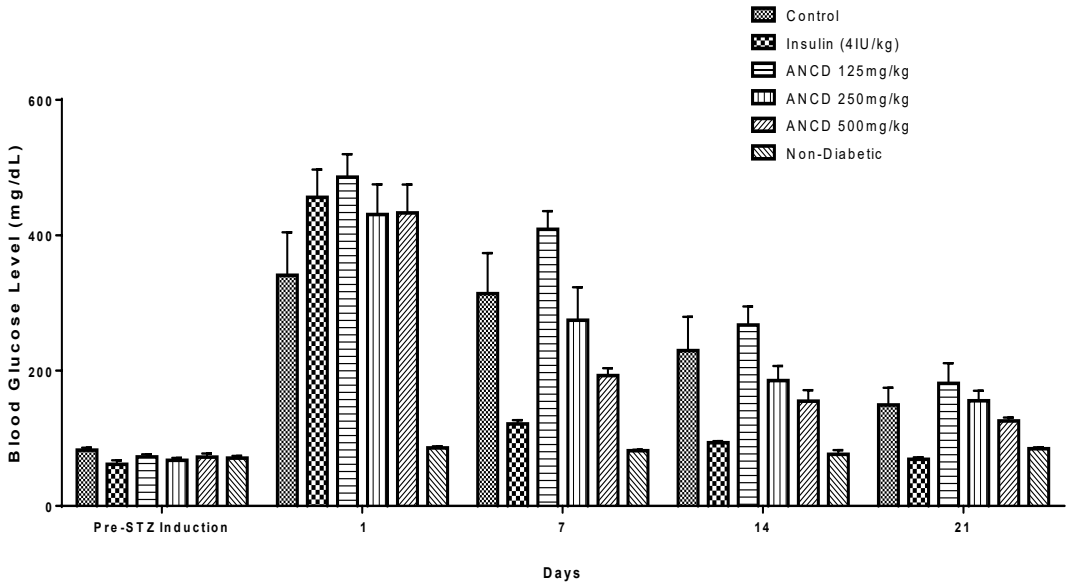
Appendix 4: Line graph of Sub-acute anti-diabetic effect of *Anchomanes difformis.*

Line graph showing the sub-acute antidiabetic effect of *Anchomanesdifformis*on STZ-induced diabetic rats. Values are expressed as Mean ± SEM (n = 6).



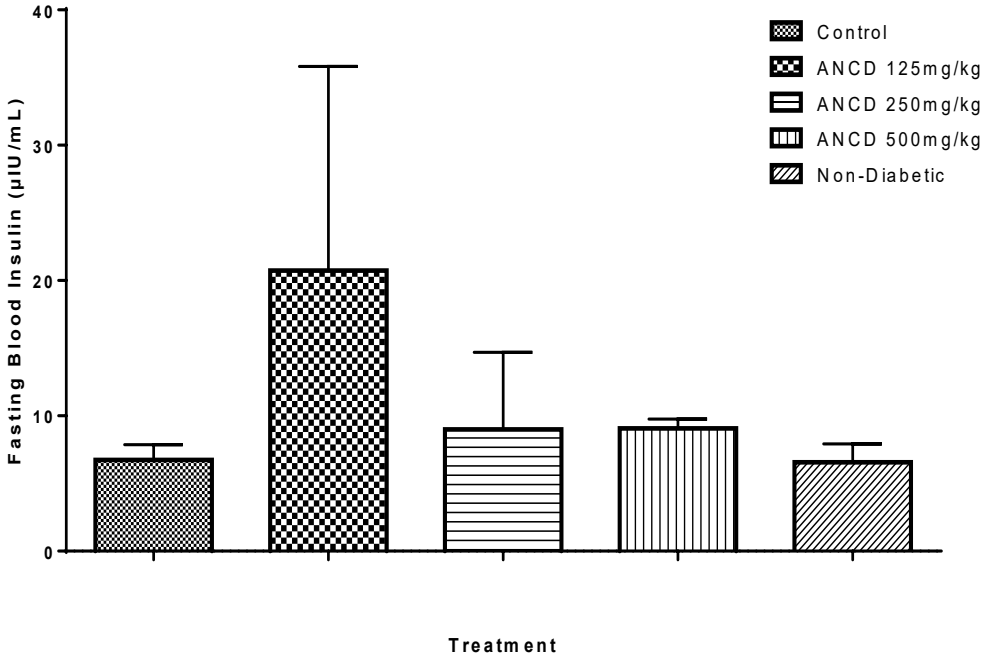
Appendix 5: Bar chart of Sub-acute anti-diabetic effect of *Anchomanes difformis.*

The sub-acute antidiabetic effect of *Anchomanesdifformis*on STZ-induced diabetic rats. Values on bar chart are expressed as Mean ± S.E.M (n = 6). *P* > 0.05. One -way Analysis of variance (ANOVA) followed by Dunnet's and Tukey's multiple comparison test.



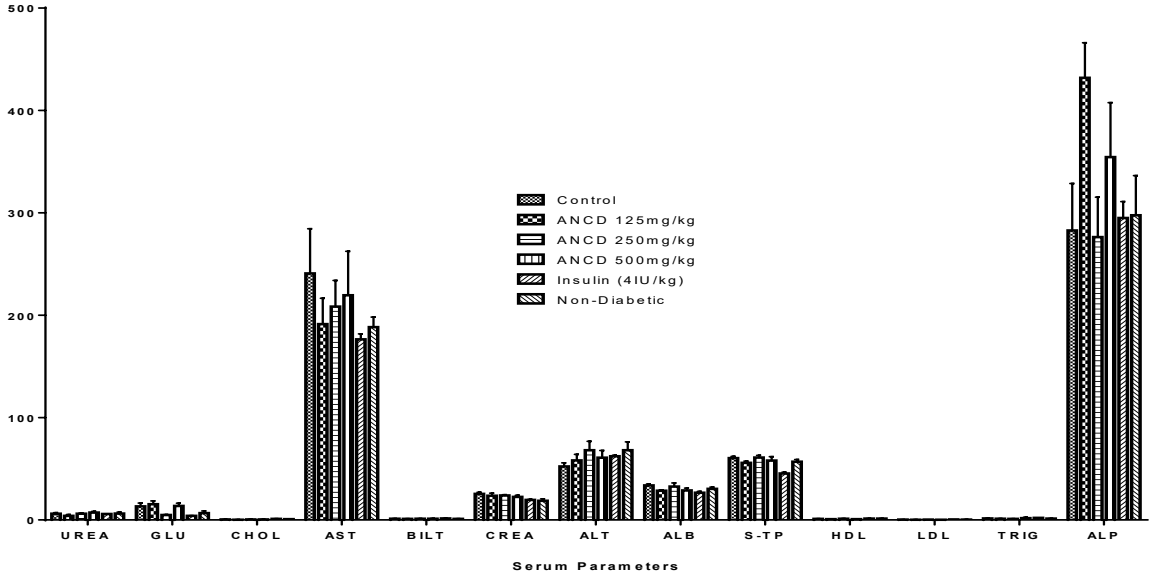
Appendix 6: Bar chart of the effect of *Anchomanes difformis* oon fasting blood insulin.

Values on bar chart are expressed as Mean ± S.E.M (n = 3). $P > 0.05$. One-way Analysis of variance (ANOVA) followed by Dunnet's and Tukey's multiple comparison test.



Appendix 7: Effect of *Anchomanes difformis* on Serum Parameter of normal non-STZ and STZ-induced diabetic rats.

Values are expressed as Mean ± S.E.M (n = 6). $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. control; $^{b}P < 0.01$, $^{d}P < 0.0001$ vs ANCD 125 mg/kg; $^{\beta}P < 0.05$ vs. 250mg/kg. (Two-way Analysis of variance (ANOVA) followed by Dunnet's and Tukey's multiple comparison test).



Chapter 5
Discussion

Acute toxicity study of the hydroethanolic extract showed the non-toxic effects when administered orally at even high doses. This may be justified by the fact that the plant used in this study is the rhizome part of the plant which is known to have high contents of carbohydrates and starch, which is relatively safe for human and animal consumption. As observed in this study, *Anchonamas difformis* did not cause a significant change in the glucose level of normoglycemic rats. This implies that the extract does not have the potential to cause hypoglycemia under normal physiologic conditions unlike some antidiabetic drugs. Insulin, which was used as one of the standards showed significant decrease in glucose levels of normoglycemic animals but there was no significant reduction in blood glucose even after the glucose was loaded. This can be explained pharmacologically by the short half-life of insulin. Glibenclamide, also a standard drug showed significant decrease in blood glucose level even after 150mins of glucose administration. In this study it was observed that *Anchonamas difformis* showed similar actions to glibenclamide. This suggests that the plant may have a mechanism of action similar to that of glibenclamide, which is a sulfonylurea. Sulfonylureas are known to act as insulin sensitizers. (That increase the secretion of insulin by the β-cells) [58]. *Anchomanes difformis* at increasing doses shows a significant reduction in glucose levels up to 420mins as was observed with glibenclamide.

In the acute study, there was significant reduction in blood glucose level observed with *Anchomanes difformis* at 125 and 500 mg/kg by the 6h after treatment. A less significant effect was also noticed at 6h post treatment in insulin treated group.

In the sub-acute study, all doses of the extract significantly reduced blood glucose levels, but a higher reduction was seen at 500mg/kg on the 7th and 14th day. In comparison to the standard drug a similar effects was noticed from the 7th to the 21st day of administration.

Due to these varying mechanisms of action, insulin profiling will go a long way in determination of whether the extract acts through rejuvenation of β cells to increase insulin levels. In this study, there was no significant increase in the level of insulin produced, ruling out its mechanism on β cells rejuvenation.

Haematological biomarkers obtainable from the blood of diabetic and normal rats which include lipid, liver enzymes, urea and creatinine assay showed a decrease in all levels of lipid profiles (HDL, LDL, Cholesterol, triglycerides and bilirubin). This can be due to the breakdown of fat for generation of glucose which causes ketoacidosis. Hence lipid and fatty acid depletion. An increase in aspartate transaminase (AST) levels was observed. AST is a non-specific hepatic biomarker because it can be elevated as a result of skeletal muscle injury or cardiac disorder. The extract increased AST level in a dose-dependent manner. This suggests a potential hepatocellular, skeletal muscle and cardiac assault. To further strengthen this view, ALP and ALT, important liver biomarkers were also increased by the extract. This suggest that *A. difformis* has hepatotoxic potentials and it should be avoided in patients with hepatic insufficiency. However, further research is needed to straighten out the odds and further clarify this view.

Conclusion

The results obtained in this study suggest that the rhizome hydroethanolic extract of *Anchomanes difformis* possesses antidiabetic activity possibly due to a combination of mechanisms, including inhibition of intestinal glucose absorption, and hepatic gluconeogenesis. The observed biological actions may be due to the presence of one or a combination phytocomponents present in the plant extract. However, due to increase in the levels of Liver biomarkers, the drug may elicit assault to the hepatic system. This requires further work to support this view.

Further studies are required to isolate, identify, and characterize the active phytoconstituents and determine the precise mechanism (s) of action.

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