Antidiabetic activity, toxicity, and phytochemical evaluation of *solanum terminale* forsk (solanaceae)

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DEPARTMENT OF PHARMACOGNOSY

ANTIDIABETIC ACTIVITY, TOXICITY, AND PHYTOCHEMICAL EVALUATION OF *SOLANUM TERMINALE* Forsk (SOLANACEAE)

By

Rajabu Mohamedi Kingo

A Dissertation Submitted in (partial) Fulfilment of the Requirements for the Degree of Master of Pharmacy (Pharmacognosy) of Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled **Antidiabetic Activity, Toxicity and Phytochemical Evaluation of *Solanum terminale* Forsk (Solanaceae),** in fulfillment of the requirements for the degree of Master of Pharmacy (Pharmacognosy) of Muhimbili University of Health and Allied Sciences.

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Prof. Sheila M. Maregesi

(Supervisor)

Date: _____

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Prof. Amos R. Mwakigonja

(Supervisor)

Date: _____

DECLARATION AND COPYRIGHT

I, **Rajabu Mohamedi Kingo**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature …… Date …….....….....

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DEDICATION

This work is dedicated to my beloved late father, Sheikh Mohamedi Mshindo Kingo who had always wished me success in my life.

ABSTRACT

Background:

Based on WHO and IDF data, prevalence of diabetes disease is on the rise. Majority of the population especially in the developing world depend on herbal medicine being their primary source for medicines or the preferred treatment. This is due to the limited access of modern conventional medicines as well as to avoid side effects associated with various oral hypoglycemic agents and insulin analogues.

Aim of the study:

This study aimed at exploring the antidiabetic, toxicity profile, phytochemical classes of compounds of *Solanum terminale* (fruits), a Tanzanian medicinal plant claimed to have hypoglycemic effects.

Methodology:

The study was experimental design. Fruits of *S. terminale* were collected from Lushoto. Identification was done using herbaria specimen and the voucher specimen was deposited at the ITM herbarium – MUHAS. Extracts were prepared by exhaustive maceration using 95% ethanol, dried in rotary evaporator and then fractionated with dichloromethane, ethyl acetate, methanol and water. Antidiabetic testing employed healthy albino mice involved in Oral Glucose Tolerance Test (OGTT) and Alloxan models. In both models, mice were treated with single doses of 100 mg/kg of extract and fractions. In alloxan model, mice were induced with diabetes by intraperitoneal injection of freshly prepared alloxan monohydrate 170 mg/kg BW, and the diabetic mice were treated daily for the period of 20 days, and FBG were recorded on day 1, 5, 10, 15 and 20. Both experiments included 5% ethanol treated mice as negative control group and chlorpropamide 100 mg/kg BW treated mice as positive control group. Acute oral toxicity followed OECD guideline using a single dose of 2000 mg/kg BW, and the treated mice were observed for 14 days for their mortality, behavioral and other changes. Before sacrifice, the weight of animals and the visceral organs were recorded, and then sent for histological evaluations. The phytochemical screening was performed by using standard

qualitative procedures of colour reactions. Results were expressed as mean ± SD, analysed by independent student's t-test, p <0.05 was considered as significant level. Ethical clearance was sought from MUHAS, IRB and animal care followed the EEC Directive of 1986; 86/609/EEC.

Results:

The extraction yield was 4.4%, while the fraction yields were 57.20, 9.35, 8.95 and 6.80% for MeOH, H_2O, EtOAc and DCM, respectively. Crude ethanolic fruit extract of *S. terminale* and fractions demonstrated clinical and statistical significant blood glucose lowering efficacy in OGTT and Alloxan models (p<0.05). Neither death nor abnormal changes in behavioral features in tested mice observed at 2000 mg/kg BW of *S. terminale* crude ethanolic fruit extract. Although the histological analysis demonstrated some organ derangements at higher dose, the fruits are probably safe for use in low doses. Tannins, cardiac glycosides, steroids, terpenoids, alkaloids, flavonoids and saponins were found to be the phytochemicals present in *S. terminale* crude ethanolic fruit extract.

Conclusion:

This study supports the claim of the traditional use of *S. terminale* fruits in diabetes management in Lushoto, especially from the crude ethanolic extract which showed activity in both OGTT and Alloxan models. Since the best activity was obtained from the crude extract, it is possible that, polar and less polar compounds are working in a synergistic manner for the reduction of blood glucose levels. As observed, the antidiabetic dose of the crude ethanolic extract i.e. 100 mg/kg BW is far much low when compared to the toxic dose tested of 2000 mg/kg BW, this plant qualifies for further work in search of antidiabetic compounds/standardization of herbal medicine.

Further work is needed including more antidiabetic screening exhausting other techniques, toxicological studies, identification of the active compounds, and standardization/formulation of *S. terminale* fruits products.

Keywords: *S. terminale*, phytoconstituents, acute oral toxicity, diabetes mellitus**.**

IKISIRI

Utangulizi:

Takwimu za Shirika la Afya Ulimwenguni na Chama cha Kimataifa kinachoshughulika na Ugonjwa wa Kisukari zinaonyesha kasi kubwa ya ongezeko la ugonjwa wa kisukari duniani, Pia idadi kubwa ya wagonjwa katika jamii ya nchi zinazoendelea hutegemea dawa za asili hasa kutoka katika mimea. Hii inatokana na wagonjwa wa kisukari kukwepa madhara yanayotokana na dawa za kisasa au upungufu wa dawa hizo kwa wagonjwa.

Dhamira ya utafiti:

Utafiti huu ulilenga kuangalia uwezo wa kushusha kiwango cha sukari iliyozidi mwilini na madhara yanayoambatana na utumiaji wa matunda ya Mjujui, inayotumika kama dawa ya kisukari na shinikizo la damu wilayani Lushoto Tanzania.

Njia:

Utafiti ulifanywa kwa njia ya majaribio ya kisayansi, kwa kutumia panya wadogo. Matunda ya Mjujui yalikusanywa Lushoto, na kutambuliwa na wataalamu wa mimea katika maktaba ya mimea "herbarium" na kumbukumbu za mmea huo zimehifadhiwa katika maktaba ya mimea ITM - MUHAS. Dawa ilitengenezwa kwa kuloweka matunda katika "Ethanol 95%". Rojo ilichujwa na kisha kukaushwa kwa kutumia kifaa maalum kiitwacho "rotary evaporator". Baada ya hapo, rojo kavu ilimumunyishwa na kupata makundi manne yafuatavyo; dichloromethane, ethyl acetate, methanol na maji.

Njia mbili za majaribio zilitumika ili kuangalia uwezo wa kushusha kiwango cha sukari kwenye damu. Katika majaribio yote Panya hakupewa chakula isipokuwa maji kwa saa 12 na kisha kiwango cha sukari kilipimwa kwa kila panya kabla hali ya kisukari haijaanzishwa. Aidha panya walipewa "5% Ethanol" kama udhibiti hasi, na dawa aina ya chlorpropamide (100 mg/kg) kama ukidhibiti chanya vipimo vikitegemea uzito wa panya.

Njia ya kwanza ilikuwa OGTT ambapo makundi ya panya yalipewa aidha 100 mg/kg ya Mjujui au 100 mg/kg za kila kimumunyisho au 5% Ethanol au Chlorpropamide (100 mg/kg) kila kipimo cha dawa kulingana na uzito wao. Baada ya nusu saa kila panya alinyweshwa

glucose kulingana na uzito wake (1 g/kg) na kisha kiwango cha sukari katika damu ya kila panya kilipimwa baada ya dakika 30, 60, 120 na 180.

Katika njia ya pili hali ya kisukari ilianzishwa kwa kutumia 170 mg/kg za Alloxan kwa njia ya sindano kila panya akipewa kipimo kulingana na uzito wake. Baada ya siku tatu kiwango cha sukari katika damu (FBG) kilipimwa na panya waliopata kisukari waligawanywa katika makundi manne na jaribio likaendelea. Kila panya alipewa aidha 100 mg/kg ya mjujui au 100 mg/kg za kimumunyisho methanol au 5% ethanol au chlorpropamide 100 mg/kg kipimo kulingana na uzito wake. Kiwango cha sukari katika damu kilipimwa siku ya 1, 5, 10, 15 na 20.

Jaribio la madhara/usumu kwa njia ya kinywa lilifuata kanuni za kimataifa za OECD kwa kutumia 2000 mg/kg ya rojo kavu, kipimo cha dawa kilitolewa kulingana uzito wa panya. Uangalizi wa karibu uliendelea kwa siku 14 kuchunguza, vifo, uzito na mabadiliko mengine ya tabia na mwili kadiri ya jedwali maalumu la OECD. Mwishoni uzito wa kila panya ulirekodiwa na kisha panya wote waliuliwa ili kupata viungo vya ndani ambavyo uzito wa kila kiungo ulirekodiwa ikifuatiwa na uchunguzi wa kihistolojia. Hatimaye majaribio ya uchunguzi wa kemikali viambata ulifanywa.

Takwimu za matokeo yaliwasilishwa kama “mean ± SD” baada ya kuchambuliwa kwa njia za “student’s t-test”, p <0.05 ilizingatiwa kama kiwango muhimu. Kibali cha maadili kilitolewa na MUHAS, utunzaji wa wanyama ulifuata Maagizo ya EEC ya 1986; 86/609/ EEC.

Matokeo:

Rojo kavu ya Mjujui ilikuwa 4.4%, iliyotoa vimumunyisho vya methanol (57.20%); H_2O (9.35%); EtOAc (8.95%) na DCM (6.80%). Rojo kavu na vimumunyisho vimeonyesha uwezo kupunguza sukari ndani ya damu katika jaribio la OGTT na Alloxan (p <0.05).

Kwa kutumia rojo kavu 2000 mg/kg, hapakuwa na kifo, mabadiliko ya tabia wala mabadiliko makubwa ya uzito kwa panya wote waliotumika katika jaribio la madhara/sumu. Pia uchunguzi wa kihistolojia haujaonyesha madhara makubwa licha ya kutumia kipimo kikubwa

cha dawa. Kemikali aina ya tannins, glycosides, steroids, terpenoids, alkaloids, flavonoids na saponins zilionekana kama kemikali viambata zilizopo katika rojo kavu ya Mjujui.

Hitimisho:

Utafiti huu umeonesha uwezo wa Mjujui kutumika kama dawa ya kisukari hususani toka kwa rojo kavu. Ikielekeza uwezekano mkubwa wa kemikali viambata kuwa na uwezo tofauti wa miyeyusho zinazofanya kazi kwa pamoja. Utafiti huu pia umeonesha hakuna madhara ya kutisha kwa viungo vya ndani katika jaribio la madhara/sumu katika panya kwa kipindi cha siku 14. Hiyo basi Mjujui inaweza kuwa dawa salama ukizingitia inaweza kupunguza kiwango cha sukari kipimo cha 100 mg/kg ukilinganisha na kipimo cha 2000 mg/kg kilichosababisha madhara kidogo. Kemikali viambata vya Mjujui vimethibitishwa kushusha viwango vya sukari kwenye damu katika machapisho yaliyopita.

Kuhalalisha matumizi ya mjujui kunahitaji tafiti zaidi zikihusisha; majaribio mengine ya madhara/sumu katika damu, vinasaba nk., pia uchunguzi wa kujua namna dawa inavyofanya kazi mwilini, kemikali husika na hatimaye utengenezaji wa dawa salama.

Maneno muhimu:
Mjujui, kemikali viambata, usumu kwa njia ya kinywa, ugojwa kwa kisukari

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ABBREVIATIONS

ANOVA	Analysis Of Variance
BW	Body weight
DCM	Dichloromethane
DM	Diabetes Mellitus
EtOAc	Ethyl acetate
FBG	Fasting Blood Glucose
g	gram
GDM	Gestational Diabetes Mellitus
H_2O	Water
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IRB	Institutional Review Board
ITM	Institute of Traditional Medicine
kg	kilogram
MeOH	Methanol
mg	milligram
min	minutes
ml	milliliters
MUHAS	Muhimbili University of Health and Allied Sciences

NCDs	Non-communicable diseases
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OECD	Organization for Economic Co-operation and Development
OGTT	Oral Glucose Tolerance Test
SD	Standard Deviation
SEM	Standard Error of the Mean
STE	Solanum Terminale Extract
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TLC	Thin Layer Chromatography
WHO	World Health Organization

DEFINITION OF TERMS

Acute oral toxicity - refers to the unintended noxious effects occurring following administration of a single dose or multiple doses given within 24 hours.

***ad libitum*-** means as one pleases or as much or as often as necessary or desired.

Diabetes mellitus – it is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia.

Dose – it is the amount of test substance administered expressed as weight of test substance per unit weight of test animal (example; mg/kg).

Hyperglycaemia – it is the state of elevated blood glucose levels.

Phytoconstituents/Phytochemical classes - are the groups of chemical compounds occurring naturally in the plants giving the medical action of the plant, also known as secondary metabolites.

Side effect - is a secondary, typically undesirable effect of a drug or medical treatment.

Toxicity – an attribute of being noxious or poisonous.

CHAPTER ONE

1. INTRODUCTION

1.1. BACKGROUND

Non-communicable diseases (NCDs) are the worldwide leading killer diseases with an estimate of 70.1% of the global deaths by 2015. In low and middle-income countries including Tanzania, the NCDs contribute to 34% of all deaths with reference to 10% in the early 80s. Among the NCDs diabetes is estimated to affect 9% of all adults worldwide [Damian *et al.*, 2017].

Diabetes mellitus is a chronic disease of metabolic disorders characterized physiologically by insufficiency in insulin secretion and/or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other apparent disorders often associated with carbohydrate, protein and lipid metabolism [Niamat *et al.*, 2012]. Modifications of lifestyle together with medical assisted weight loss programs, diabetes education about diet, physical activity, use of oral hypoglycaemic agents such as sulfonylureas, meglitinides, biguanides, etc., use of insulin and blood glucose monitoring education are the management options used to obtain the glycaemic control [Reusch and Manson, 2017].

Weight gain, hypoglycaemia, edema, anaemia, nausea, and diarrhoea are among of the reported prominent side effects from the pharmacotherapeutic option of the diabetes management [Raj *et al.*, 2016].

Plants have formed the foundation of traditional medicine systems that have been in existence for years and they are still used to provide mankind with new remedies. Research on management of diabetes using traditional medicinal plants has brought scientists' focus, as various plants being used for managing diabetes traditionally confirmed their blood glucose lowering efficacy through different *in vitro* and *in vivo* models [Day and Bailey, 2006]. Extracts of some plants are used to treat diabetes in traditional medicine and have scientifically been proven to have antidiabetic activity [Osadebe *et al.*, 2014].

The use of plants in treatment of diabetes is due to their ability to offer antihyperglycaemic effect through different mechanisms. Among of such mechanisms include; increasing insulin secretions, enhancing glucose utilization, reducing the rate and speed of intestinal glucose absorption [Kooti *et al.*, 2016]. Moreover, plants also reduces blood glucose levels by eliminating free radicals, lowering cholesterol levels and regulating the glycemic metabolism making them more attractive substitutes for modern antidiabetic drugs [Bedekar *et al.*, 2010 ; Choi and Roh, 2011].

Among the reported plants used in diabetes management include; *Acacia tortilis, Diospyros usambarensis, Ehretia amoena, Lannea schimperi, Moringa oleifera, Cyphostemma* spp., *Bridelia micrantha,* and *Cymbopogon citratus* [Moshi and Mbwambo, 2002]; *Acacia brispica, Albizia anthelmintica, Boscia salicifolia, Cassia singueana, Bridelia carthatica, Centella asiatica, Dombeya rotundifolia, Ficus capensis, Grewia bicolor, Hibiscus micrantha, Catunaregan obovata, Rumex usambarensis, Waltheria indica, Ximenia americana* etc. Others are the roots of *Afzelia quanzensis* and *Bridelia duvigneaudii,* fruits of *Cyphomandra crassifolia,* tubers of *Dioscorea praehenilis* and stem bark of *Ficus fischeri* [Moshi *et al.*, 2006; Moshi *et al.*, 2012]. On the other hand safety evaluation is in most cases never done parallel to the establishment of antidiabetic activity and the isolation and identification of antidiabetic compounds are few.

A number of compounds have been isolated from plants and become useful clinical agents to be in use to date [Kong *et al.*, 2003]. Some of the modern antidiabetic medicines originated from natural sources; for example, metformin which was derived from galegine, a hypoglycemic component isolated from *Galega officinalis*, a plant used in traditional medicine to treat diabetes [Trojan-Rodrigues *et al.*, 2012]. This suggests an enormous possibility that still exists for the discovery of many novel drugs from medicinal plants and/or enabling standardization of herbal products.

Solanum terminale, the least studied plant in the genus *Solanum,* its fruits are mainly used traditionally for management of diabetes and hypertension in Lushoto, Tanzania. The plant to the best of our knowledge, has never been scientifically reported to have antidiabetic efficacy,

rather it has been reported to be used for kwashiorkor treatment in south-west Nigeria [Lawal *et al.*, 2010] and to induce labour during childbirth in western Uganda [Kamatenesi-mugisha and Oryem-origa, 2007]. This study focused on assessing the antidiabetic activity, toxicity and phytochemical evaluation of *S. terminale* fruits as one of the many unstudied Tanzanian medicinal plants claimed to treat diabetes.

1.1.1. Plant Description

Solanum terminale is a creeper or climber to 3(-7) m. Leaves are elliptic, 7(-11) × 3(-6) cm, and sparsely pubescent to hairless. The leaf base is rounded, decurrent on the petiole; apex acuminate; entire margin and petiole is 1-2 cm long. Flowers composed of 3-5 pedunculate umbels arranged in a dense terminal inflorescence. Pedicels 1-2 cm, thickened below the flower. Calyx lobes 5. Petals 7-10 mm, white, mauve, purple or blue; anthers yellow. Flowers 15-20 mm in diameter. Fruit 6-10 mm in diameter, spherical, red when ripe.

The plant is distributed Throughout Africa from Guinea eastwards to Ethiopia and south to South Africa; also Yemen and the Comoro Islands [Flora of Zimbabwe].

(a)

(b)

Figure 1: Image of *S. terminale* [Source (a) – Flora of Zimbabwe, (b) – Kingo]

1.2. PROBLEM STATEMENT

Based on the increasing growth of diabetes prevalence worldwide [IDF, 2017] together with the lifestyle modification, there is a need for searching of the new antidiabetic molecule(s) with fewer side effects and/or with a novel mechanism of action(s) to enable its management from natural sources, especially plants.

To date, about a hundred and fifty (150) Tanzanian medicinal plants have been documented for management/treatment of diabetes in six ethnomedical surveys conducted in a small portion of Tanzania, but very few plants have been subjected on preliminary antidiabetic testing [Moshi *et al.*, 2000; Moshi and Mbwambo, 2002; Augustino *et al.*, 2011; Moshi *et al.*, 2012; Lunyera *et al.*, 2016; Credo *et al.*, 2018].

There is the generalised fallacy in the community on the safety of medicinal plants for management of various diseases, since they are from nature, while it is contrary to the reality as various adverse effects have been reported from the use of plants as medicine.

Solanum terminale, being the unstudied plant used traditionally for diabetes management qualified for the purpose. Thus, this stimulated the need for screening and investigation of *S. terminale* fruits as potential sources of new antidiabetic compounds for primary health care, through detailed scientific studies that will enable its incorporation in modern medicine and/or standardization of herbal medicine to solve the diabetes treatment using safe medicines.

1.3. RATIONALE OF THE STUDY

The study was intended to provide scientific data on the antidiabetic activity, safety profile, the phytochemical groups and active fraction(s) for further work on isolation of anti-diabetic compound(s) required for drug discovery and development, and/or preparation of the safe standardized antidiabetic herbal medicine.

1.4. RESEARCH QUESTIONS

The study was based on the following questions;

i. Do the fruits of *S. terminale* possess antidiabetic activity?
ii. Are the fruits safe when taken orally?
iii. What are the phytochemicals present in the *S. terminale* fruits?

1.5. BROAD OBJECTIVE

The main objective of the study was to investigate the antidiabetic activity, toxicity and phytochemical contents of *S. terminale* fruits.

1.6. SPECIFIC OBJECTIVES

The specific objectives were:

i. To determine for the antidiabetic activity of extract and fractions of *S. terminale* fruits.
ii. To determine acute oral toxicity of *S. terminale* fruits.
iii. To determine phytochemical classes of crude ethanolic extract of *S. terminale*.

1.7. LITERATURE REVIEW

1.7.1. Diabetes Mellitus

Diabetes mellitus, the most common endocrine disorder is a chronic metabolic disorder, which occurs when there is absolute and/or relative decrease in insulin secretion and/or when the body cannot use insulin in an effective way leading to an increase in blood glucose levels [Radenković *et al.,* 2016]. The pancreas produces insulin which is an essential hormone responsible for transportation of glucose from the bloodstream into the body cells where the glucose is transformed to energy. Absence/inadequate amount of insulin or insensitivity of the body cells to respond to insulin facilitate the elevation of blood glucose level (hyperglycaemia) which is the key indicator of diabetes [WHO Expert Committee, 1999].

This collective disorder which when not properly managed and treated facilitates severe complications which raise the cost of care and affects the patient's quality of life [Rios *et al.*, 2015]. These complications including; macrovascular complications like cardiovascular diseases and microvascular complications like blindness and foot ulcers [West *et al.*, 2016]. Thus, management of the diabetes mellitus must be followed to prevent and/or delay such problems [IDF, 2017].

Diabetes mellitus is categorized into various subtypes; the main being the Type I, T1DM (insulin dependent diabetes mellitus, IDDM), the Type II, T2DM (non-insulin dependent diabetes mellitus, NIDDM) and gestational diabetes mellitus (GDM). Almost 90% of the diabetic patients in any country have the T2DM, hence, the commonest form of diabetes worldwide [Bedekar *et al.*, 2010]. Other non-common forms/types of diabetes mellitus include monogenic diabetes and secondary diabetes [IDF, 2017].

1.7.2. Diabetes Prevalence

There has been a significant rise in the global and regional prevalence of diabetes and impaired glucose tolerance associated with the rapid urbanization and sedentary lifestyle. The global prevalence of diabetes estimation had tripled from the year 2000 to 2017 i.e. from 151 million to 451 million diabetic cases [IDF, 2017]. Worse still, diabetic reported cases are expected to reach 693 million by 2045 [Cho *et al.*, 2018].

In 2013, about 19.8 million people in Africa were estimated to have diabetes, and approximately 75% was still undiagnosed. The pre-diabetes prevalence in sub-Saharan Africa was 8.3% and expected to rise to 9.3% by 2035 [IDF, 2013]. It was also reported that diabetic cases exceed 40 million cases by 2045 in sub-Saharan Africa [Renzaho, 2015]. African countries with the highest number of diabetes patients are Nigeria, South Africa, Ethiopia and Tanzania with 3.9 million, 2.6 million, 1.9 million and 1.7 million diabetic cases, respectively [IDF, 2013].

The diabetic prevalence increase in sub-Saharan Africa has been facilitated by the rise of obesity and other cardiovascular risk factors [Chiwanga *et al.*, 2016]. Unfortunately, health

systems and infrastructure, and biomedical care are still under capacity to meet the needs of diabetes patients in middle and low-income countries [Lunyera *et al.,* 2016].

1.7.3. Risk Factors for Diabetes

Among the risk factors for diabetes, literature has reported obesity to be a major modifiable risk factor for type 2 diabetes [Damian *et al.*, 2017]. Other factors associated with development of diabetes include increased age, physical inactivity, genetical susceptibility (family history of the disease) [American Diabetes Association, 2014], autoimmune diseases like Addison's disease [Ozougwu *et al.*, 2013], and women with history of gestational diabetes [Fetita *et al.*, 2006].

1.7.4. Management of Diabetes mellitus

Diabetes mellitus management comprises the complex matrix of physical activities, dietary and pharmacotherapy options [Choi and Roh, 2011]. The lifestyle modifications of diabetic patients help in lowering the glycemic concentrations and improve the frequently coexisting risk factors for cardiovascular disease. Attaining adequate glycemic control with lifestyle modification alone is unachievable for the majority of diabetic patients, hence the need for pharmacotherapeutic agents [Inzucchi, 2002].

Glycaemic control is the central point in the prevention of diabetic microvascular diseases such as retinopathy, nephropathy, and neuropathy whereas diabetic macrovascular complications such as myocardial infarction prevention needs the control of the typical cardiovascular risk factors which embraces insulin resistance [DeFronzo *et al.*, 2015].

Type 1 DM necessitates the uninterrupted supply of high-quality insulin therapy. Type 2 DM management and other forms of diabetes need the use of oral hypoglycemic agents as pharmacotherapeutic option, but when in extreme or failure of oral hypoglycemic agents, diet and physical activities, insulin therapy should be used [IDF, 2017]. Nevertheless, side effects and the failure rate of the medicines used in the treatment of diabetes in preventing the long term complications of diabetes decreases patient tolerance on the use. Therefore, there is a

need for new therapeutic modalities with improved diabetes management, and better patients tolerance [Choi and Roh, 2011].

1.7.5. Modern Medicine for Diabetes

Different approaches have been established for the treatment of diabetes as to up to date i.e., insulin therapy, oral hypoglycemic therapy, nutritive therapy, and/or lifestyle modification [Kooti *et al.*, 2016]. The oral hypoglycemic agents (OHAs) act through various mechanisms, including stimulation of insulin secretion (sulfonylureas and meglitinides), increasing peripheral glucose absorption (biguanides and thiazolidinediones), delaying carbohydrates absorption from the intestine (α-glucosidase inhibitors) and reduction of gluconeogenesis (biguanides). They can either be used alone or in combinations with other oral hypoglycemic agents and/or insulin [Rani and Kumar, 2015].

Since the disease involves multiple pathophysiologies, a monotherapy would not be able to sufficiently reverse the multiple anomalies of the disorder; hence a combination therapy received extensive acceptance and expected to grow further [DeFronzo *et al.*, 2015].

The therapeutic potential of these synthetic antidiabetic drugs is undoubted, but they are associated with some drawbacks which include fluid retention, hypoglycemia at higher doses, hepatic problems, potential cardiac hypertrophy, lactic acidosis, and weight gain. Also, it has been reported on damaging effects on beta cell function and insulin action (glucotoxicity) caused hyperglycaemia *per se* [Rani and Kumar, 2015]. Combined intensive efforts are required for searching more effective drugs for the treatment of T2DM that are safe to overcome the undesirable side effects of synthetic drugs.

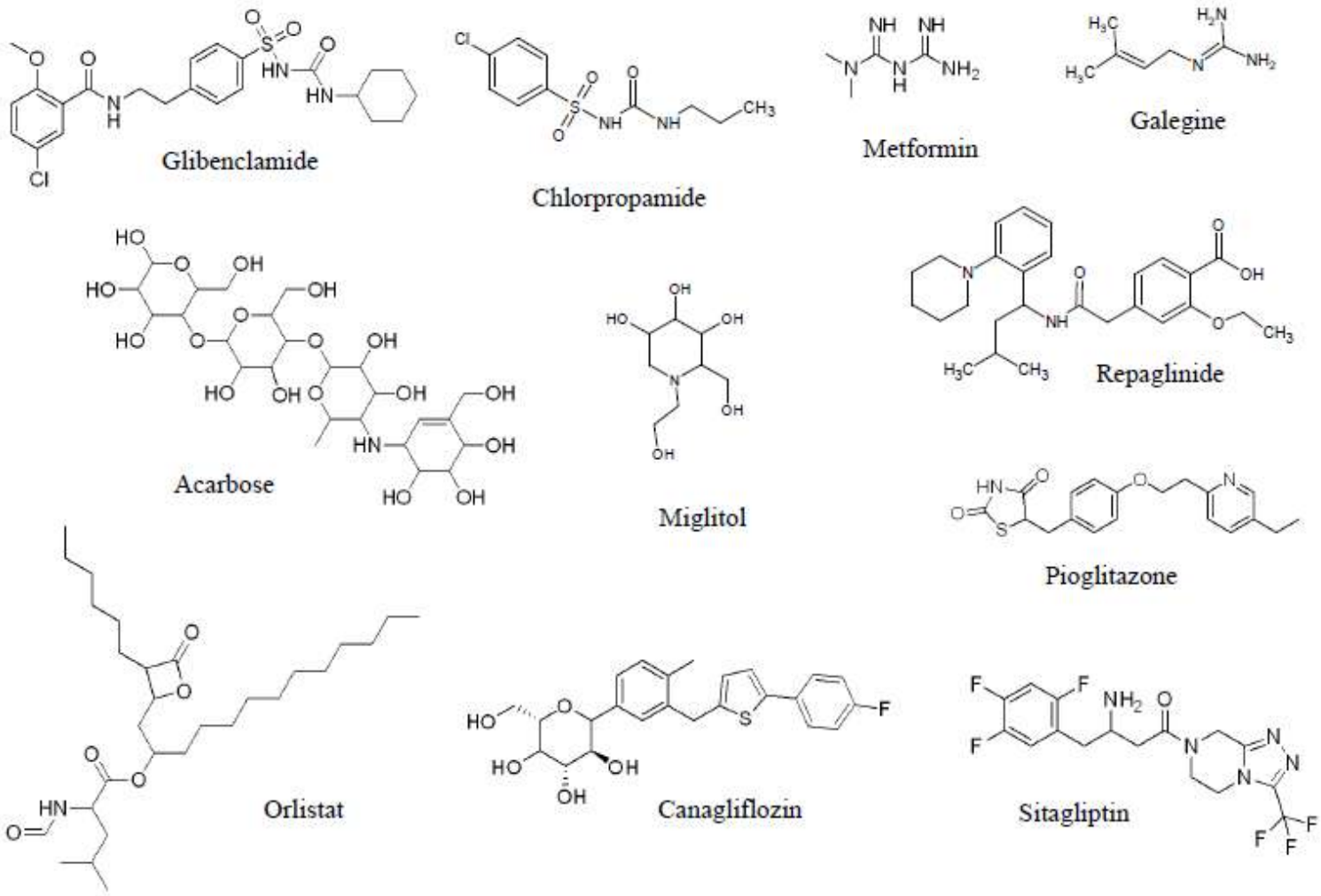

Figure 2: Some chemical agents having antidiabetic activity

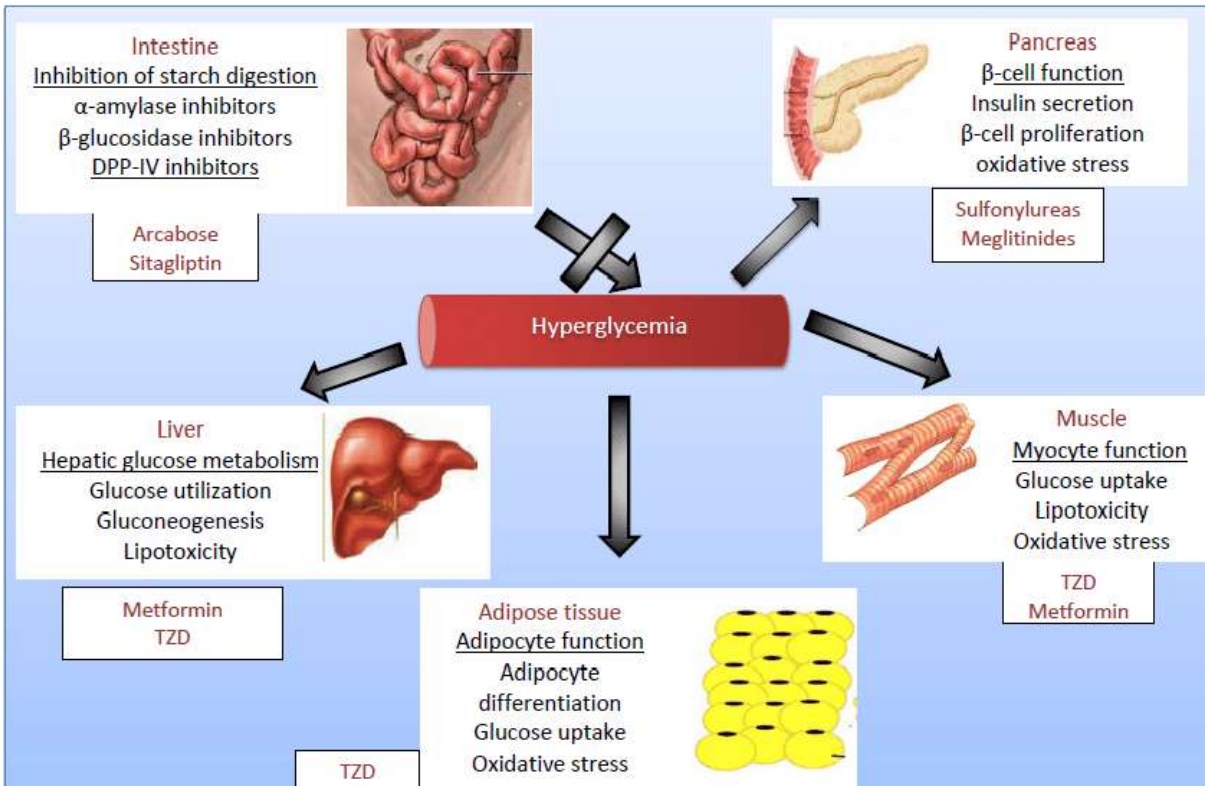

Figure 3: Pharmacological Targets for Diabetes Management

1.7.6. Side Effects of Synthetic Antidiabetic Medications

Nevertheless, current oral hypoglycemic agents used in modern medicine are associated with various side effects necessitating patients to search for alternative therapies mainly herbal medicines. A recent study in Nepal consisting of 183 participants, showed that 30.6% suffered from various side effects including hypoglycemia, dizziness, fatigue, joint pain, eye irritation, tingling sensation and gastrointestinal disorders like abdominal discomfort and diarrhoea. Hypoglycemia predominated with 49.7% of the reported cases [Manandhar Shrestha *et al.*, 2017]; this is the leading fact making a majority of the population to opt traditional means of treatment as most are not related to the said side effects.

1.7.7. Traditional Medicinal Plants

Despite substantial efforts in developing synthetic medicines in these past three decades, the treatment outcome of diabetes is not totally successful and desirable. There are various disadvantages associated with the use of these conventional antidiabetic medicines such as serious side effects, contraindications, reduction of drug's efficiency on disease progression and even toxicity. These highpoint the need for novel therapies that decrease the rate of recurrence and severity of DM exacerbations [Kooti *et al.,* 2016]. The resulting outcome is the growing concern in phytomedicine and various extracts are being studied the purpose of identifying their antidiabetic mechanism of action, safety and efficacy [Dsouza and Lakshmidevi, 2015]. WHO supported medicinal plants' evaluation focusing on their efficacy, affordability and being of little or no adverse effects [Balogun *et al.,* 2016].

Medicinal plants have been used in decades for management of various diseases. The use of plants in treatment of diabetes is due to their ability to offer antihyperglycaemic effect by various mechanisms. Such mechanisms include; improving the performance of pancreatic tissue by increasing insulin secretions, raising of the gastrointestinal contents viscosity thereby slowing the gastric emptying time and acting as a barrier to diffusion, enhancing glucose utilization, reducing the rate and speed of intestinal glucose absorption through alteration of the fiber content [Rani and Kumar, 2015; Kooti *et al.*, 2016], eliminating free radicals, lowering cholesterol levels and regulating the glycemic metabolism [Choi and Roh, 2011].

About 1200 plants have been reported to be in use traditionally for diabetes management worldwide, however majority of them lack scientific proof [Hsu *et al.*, 2009] for their use. Reports in the literature indicate these plants to be efficacious in the treatment of diabetes and associated symptoms with few side effects in comparison to the current antidiabetic medications [Choi *et al.,* 2015]. Continued investigations of antidiabetic plants, has resulted in a report of approximately 400 plants having *in vitro* and *in vivo* antidiabetic properties [Chang *et al.,* 2013].

Some of the commonly used plants for management of diabetes in Africa include *Bridelia ferruginea, Herichrysum petiolare, Gongronema latifolium, Brachylaena discolor, Dioscorea dumentorum, Artemisia afra, Bulbine natalensis,* and *Vernonia amygdalina*. For some of these plants, hypoglycaemic effects have been demonstrated in some experimental model of diabetes [Erasto *et al.*, 2006; Akah *et al.*, 2011].

Some of the modern antidiabetic medicines originated from natural sources especially plants. For example, metformin was derived from a hypoglycemic component galegine (see **Figure 2**), isolated from French lilac tree, *Galega officinalis*. This plant is used in traditional medicine to treat diabetes [Grover *et al.*, 2002].

Various functional foods in different forms such as fruits and vegetables have been reported to be used in the management of diabetes. For example, the investigations on the pumpkin-rich diet have proven that it has pharmacological activity in reducing blood glucose levels [Adams *et al.*, 2011].

Moreover, the use of polyherbal preparations having antidiabetic effect is also evident due to the incorporation of agents with diverse mechanisms of action with agonistic/antagonistic, potentiative and/or synergistic actions. The purpose is to achieve the maximum therapeutic efficacy with fewer side effects [Farag *et al.*, 2014]. Shah *et al*., (2019) reported the significant decrease in the elevated blood glucose levels with the use of the polyherbal formulation comprising of *Gymnema sylvestre* (Leaves), *Trigonella foenum-graecum* (Seeds) and *Phyllanthus emblica* (Fruits) on different proportions in streptozotocin induced diabetic rats [Shah *et al.*, 2019]. Since herbal formulations containing multiple plants are considered to have greater effects than the individual herbs taken separately, many polyherbal preparations in use have been documented [Ghorbani, 2014].

1.7.8. Traditional Antidiabetic Medicinal Plants in Tanzania

Few systematic antidiabetic ethnobotanical studies have been conducted in Tanzania. Some of the reported plants used for treatment of diabetes and diabetes-associated symptoms include *Boscia salicifolia, Ehretia amoena, Dombeya rotundifolia, Grewia bicolor, Lannea*

schimperi, Cyphostemma spp., *Diospyros usambarensis, Ficus capensis, Acacia tortilis,* and *Cymbopogon citratus* [Moshi and Mbwambo, 2002]. Others include *Acacia brispica, Catunaregan obovata, Albizia anthelmintica, Moringa oleifera, Hibiscus micrantha, Cassia singueana, Rumex usambarensis, Bridelia carthatica, Centella asiatica, Bridelia micrantha, Waltheria indica, Ximenia americana,* roots of *Afzelia quanzensis* and *Bridelia duvigneaudii,* fruits of *Cyphomandra crassifolia,* tubers of *Dioscorea praehenilis* and stem bark of *Ficus fischeri* [Moshi *et al.*, 2006; Moshi *et al.*, 2012].

In vivo analysis of some Tanzanian medicinal plants revealed the presence of antidiabetic activity, for example; *Phyllanthus amarus* and *Securinega virosa* that demonstrated activity in a dose-dependent manner by lowering of the area under OGTT curve between 100 mg/kg BW and 1000 mg/kg BW [Moshi *et al.*, 2000]. In a recent study, roots extract of *Bridelia duvigneaudii* was able to lower the blood glucose levels of the tested albino mice (OGTT) at a dose of 200 mg/kg BW [Credo *et al.*, 2018]. These, plus other studies, gave an overview of the potential of Tanzanian medicinal plants in managing diabetes, as they might be further analysed and incorporated in drug discovery and/or development of standardized herbal products.

In this study, the fruits of *Solanum terminale* were evaluated for its potential antidiabetic activity. The genus *Solanum* L. (Solanaceae), comprises about 1400 species of flowering plants distributed in tropical and temperate zones [Parasuraman *et al.*, 2018].

Common edible plants such as potato (*S. tuberosum*), tomato (*S. lycopersicum*) and eggplant (*S. melongena*) belong to this genus, while some of its species are poisonous others are medicinally useful [Weese and Bohs, 2007]. The medicinal properties of these plants are due to the presence of various phytochemicals among which are steroidal glycoalkaloids, polyphenols, alkaloids, flavonoids, steroids, saponins, etc. [Gnana *et al.*, 2013; Braguini *et al.*, 2018].

Several compounds have been isolated from genus *Solanum*, including esculetin, epigenin, coumarin, methyl caffeate, campesterol, stigmasterol, lupeol, diosgenin, solasodine,

tomatidenol, oleic acid, stearic acid, linoleic acid, leucine, quercetin, astorvosides A-G, solanolides, torvanol A, kaempferol, rutin, rhamnose, etc. [Yousaf *et al.*, 2013] which are responsible for their array of biological activities.

S. xanthocarpum fruits [Kar *et al.*, 2006], *S. anguivi* fruits [Elekofehinti *et al.*, 2013], *S. torvum* fruits [Gandhi *et al.*, 2011], *S. lycocarpum* fruits [Farina *et al.*, 2010], *S. melongena* fruits [Kwon *et al.*, 2008], *S. trilobatum* leaves [Doss *et al.*, 2009] and both leaves and berries of *S. nigrum* [Umamageswari *et al.*, 2017] are among the plants from the genus *Solanum* reported to have hypoglycemic and/or antidiabetic activity in animal experiments.

Fresh fruits of *S. terminale* locally known as Mjujui (Sambaa) in Lushoto district located in the North Eastern highlands of Tanzania are claimed to manage/treat diabetes and hypertension. Little is documented of the plant except for the treatment of kwashiorkor in south-west Nigeria [Lawal *et al.*, 2010] and to induce labour during childbirth in western Uganda [Kamatenesi-mugisha and Oryem-origa, 2007]. To clear out the doubt, it is very essential to establish the scientific evidence on the hypoglycaemic claims of *S. terminale* fruits from the local residents, thus this study came with the preliminary results regarding the claim.

1.7.9. Medicinal Plants Safety

It is generally assumed that plants are the safe option for the management of various diseases since they are natural. This generalization is not true since some plants are potentially toxic or may cause side effects when consumed [Muntean *et al.*, 2016]. Various toxicity/side effects have been reported in various studies especially the hepatotoxicity, other cases being kidney diseases, dermatologic effects, neurological effects, cardiovascular problems, etc., from the use of various medicinal plants worldwide [Saad *et al.*, 2017]. On the other hand, some plants do not exhibit acute toxicity at normal (lower) doses, for example, no side effect is observed at the dose under 270 mg/kg BW of the *S. nigrum* leaf extract, but it causes significant organs damage on kidney, intestines, lungs, and heart on higher doses (8640 mg/kg BW) [Kasali *et al.*, 2016].

Also, a study conducted in Uganda showed an association between the use of traditional medicines and the increase in liver fibrosis [Auerbach *et al.*, 2012]. There is the need to establish safety profiles of the plants used medicinally. This will to save the majority of the population who are using these plants including *S. terminale*, as their primary means of management on their various ailments.

Moringa oleifera ethanolic leaf extract has been revealed to have the oral LD_{50} value greater than 5000 mg/kg BW, hence it is considered practically non-toxic which implies that the extract can be consumed at higher doses without fear of toxicity [Idakwoji *et al.*, 2015].

Acute oral toxicity studies of some other *Solanum* species have been reported, for example, the oral LD_{50} value of *S. nigrum* extract was estimated to be 3129 mg/kg BW and regarded safe [Son and Yen, 2014] whereas *S. cernuum* hydroalcoholic extract was reported to have the oral LD_{50} value of 14.50 g/kg BW [Almanca *et al.*, 2011].

Solanum terminale fruits are among of the medicinal plants used locally for management of diabetes but lack the scientific evidence to support the claims. This study aimed at testing the hypoglycemic activity of *S. terminale* fruits, safety and determination of the phytochemical groups of compounds attributing to their antidiabetic actions.

1.7.10. Methods for Antidiabetic Activity Screening

Antidiabetic screening can be performed through *in vitro* and *in vivo* techniques. The procedures are important for the antidiabetic new drug discovery and development process [Karthikeyan and Balasubramanian, 2016], for example *in vitro* tests serves to establish mechanisms and definitive toxicities, while *in vivo* tests demonstrate how such mechanisms perform under clinical or pathological state [Dsouza and Lakshmidevi, 2015].

The *in vitro* antidiabetic screening tests involves *in vitro* studies on insulin secretion and *in vitro* studies on glucose uptake [Fröde and Medeiros, 2008]. For example incretins, PPAR, amylase inhibition, α-glucosidase enzyme inhibition, protein tyrosine phosphatase 1B inhibition, cell lines assays, etc. [Karthikeyan and Balasubramanian, 2016].

Chemical, surgical, and genetic modifications are the *in vivo* antidiabetic screening models in use. Alloxan and streptozocin are the chemical diabetogenic agents used for antidiabetic screening procedures, total or partial pancreatectomy in animals is a surgical procedure leading to diabetic animal for testing and spontaneous induced genetic mutations of diabetic animals' type 2 diabetes transmitted from generation to generation [Fröde and Medeiros, 2008].

1.7.11. Significance of *In Vivo* Techniques in Diabetic Research

Diabetes develops as a multiple pathophysiology disorder, of which those affected regions are the potential targets for drug development and subsequent management of the disorder. Most of the antidiabetic plant studies are based on using *in vitro* methods. The drawback with this approach is the difficulty on finding new bioactive compounds due to the testing that involves a certain enzyme, metabolic pathway or single cell types, thereby reducing significantly the identification of antidiabetic plant extract or compounds [van de Venter *et al.*, 2008]. Furthermore, they measure only immediate or acute effects missing the delayed outcome that requires chronic exposure of the antidiabetic compounds [van de Venter *et al.*, 2008]. Considering the latter, *in vivo* animal models are more appropriate for the evaluation of anti-diabetic medicinal plants [Afolayan and Sunmonu, 2010].

1.7.12. Chemical Diabetogenic Agents

There are various chemical compounds in use for induction of experimental diabetes, but the most used is alloxan (31%) and streptozotocin (69%) mainly administered intravenously, intraperitoneally or subcutaneously, and the dose for diabetes induction depends on several factors such as route of administration, animal species and nutritional status [Kumar *et al.*, 2012].

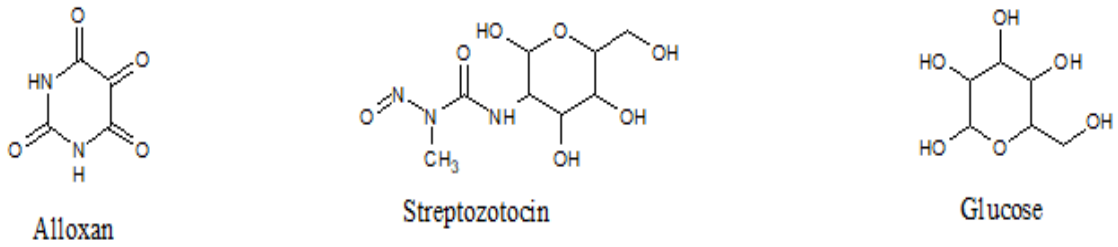

Figure 4: Structure of glucose and some of its analogues used in diabetes induction

Alloxan and streptozotocin act through different pathways. Alloxan causes diabetes by two mechanisms, namely inhibition of glucokinase resulting to selectively inhibition of glucose-induced insulin secretion, and induction of the reactive oxygen species (ROS) formation leading to selective necrosis of beta cells. Whereas, streptozotocin causes diabetes by inhibition of insulin secretion and a state of insulin-dependent diabetes mellitus due to its alkylation potency [Lenzen, 2008]. Other diabetogenic compounds in use include dithizone, vacor, monosodium glutamate, goldthioglucose and 8-hydroxyquinolone [Radenković *et al.*, 2016).

Alloxan somewhat shares structural similarity to glucose, and its movement across the plasma membrane to beta cells is also carried out by GLUT2 as glucose, hence competitive inhibition could occur upon simultaneous administration [Ighodaro *et al.*, 2017]. Therefore, prior to Alloxan administration to test animals, prolonged fasting is encouraged to avoid competition to receptor.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. STUDY DESIGN

The study was an experimental interventional design.

2.2. EXPERIMENTAL ANIMALS AND SAMPLE SELECTION

Theiler white albino mice were used in this study; both females and males were randomly selected aged 8 – 12 weeks.

2.3. MATERIALS

Glucometer and testing strips (GlucoPlus Inc., Canada), albino mice and animal cages (Animal house - MUHAS), Digital weighing scale (Escali, USA), standard animal feeds pellets (Hill pack ltd, Dar es salaam, Tanzania), 1 ml syringes, gloves, masks, aluminum foils, and cotton wools (Local Pharmacies, Dar es salaam, Tanzania) were used for various purposes in the study.

2.4. CHEMICALS AND REAGENTS

Methylated spirits, 95% ethanol, iron (III) chloride, chloroform, hydrochloric acid, normal saline, dichloromethane (Scan Tanzania ltd, DSM, Tanzania), ethyl acetate, distilled water, concentrated sulphuric acid, mercuric chloride, lead acetate, acetic acid, methanol, and chlorpropamide tablets (Dibonis®, Cosmos Ltd., Kenya) were bought from local suppliers and pharmacies. All reagents used were of analytical grade. Alloxan monohydrate was donated by Prof. Luc Pieters of the University of Antwerp, Belgium.

2.5. PLANTS COLLECTION AND IDENTIFICATION

Following botanical collection procedures, *S. terminale* fruits were collected from Lushoto in November 2018. Identification was done using herbaria specimen by the senior botanist and the voucher specimen were deposited at the ITM herbarium – MUHAS and Pharmacognosy Department, MUHAS.

2.6. EXTRACTS PREPARATION

Fresh pounded *S. terminale* fruits (800 g) were exhaustively extracted with 95% ethanol by maceration. The extract was dried using the rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 40° temperature to reduce the solvent to the maximum. Then, the extract was further dried by freeze-drying at very low temperature and pressure (Edwards High Vacuum International Crawley, Sussex, England).

2.7. FRACTIONATION

The crude ethanolic extract (20 g) was adsorbed on 40 g of silica gel and packed on a column. To obtain four fractions, the column was eluted under gravity with the following solvents, dichloromethane, ethyl acetate, methanol, and water. During the process, elution for each solvent was exhaustive (checked by TLC spotting).

2.8. YIELD CALCULATIONS

The percentage yield for the crude ethanolic extract was calculated using this formula:

$$\%\ yield\ of\ crude\ extract = \frac{mass\ of\ the\ crude\ extract\ (g)}{mass\ of\ the\ plant\ material\ (g)} \times 100$$

The fractions percentage yield the formula used were;

$$\%\ yield\ of\ fraction = \frac{mass\ of\ the\ fraction\ (g)}{mass\ of\ the\ crude\ extract\ (g)} \times 100$$

2.9. ANTIDIABETIC TESTING

2.9.1. Selection of animals for antidiabetic testing

Experiments were performed using healthy young adult albino mice, weighing 20-32 g, aged 8 to 12 weeks old. Both male and female were used, females being nulliparous and non-pregnant. The animals were housed in metal cages, allowed to feed *ad libitum* and lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by test substance and dose. All mice were acclimatized for 4 - 7 days prior to the test.

The mice were randomly divided into groups for the OGTT and Alloxan-induced diabetic mice each group containing 5 mice in OGTT and 8 mice in alloxan model testing. Each mouse in each group was identified by the markings using permanent marker (colours) on various body parts for easy identification of the received dose.

Figure 5: Picture showing some coloured mice involved in the study

2.9.2. Evaluation of oral glucose tolerance test (OGTT)

The crude extract was administered orally in a single dose by using specially designed mice oral needle, gavage. Prior to experiment the fasting blood glucose was recorded for each mouse just before treatment with 5% ethanol in normal saline [Gad *et al.*, 2006] as negative control and vehicle, extract/fractions (at 100 mg/kg BW) or chlorpropamide 100 mg/kg BW, and 30 minutes later an oral glucose load of 1 g/kg BW were given to each mouse.

The blood glucose levels for each mouse was determined using a glucometer at 0.5, 1, 2, and 3 h after the oral glucose load and recorded accurately [Credo *et al.*, 2018].

Mice for OGTT were grouped as follows;

Group 1: Negative control - mice administered with 5% ethanol 10 ml/kg BW

Group 2: mice administered with the crude ethanolic extract at the dose of 100 mg/kg BW

Group 3: mice administered with the dichloromethane fraction at the dose of 100 mg/kg BW

Group 4: mice administered with the ethyl acetate fraction at the dose of 100 mg/kg BW

Group 5: mice administered with the methanol fraction at the dose of 100 mg/kg BW

Group 6: mice administered with the water fraction at the dose of 100 mg/kg BW

Group 7: Positive control - mice administered with chlorpropamide 100 mg/kg BW

2.9.3. Induction of diabetes

Prior to the induction of diabetes, mice fasted for 14 hours. Fasting is followed by measuring their weight and blood sample collection from the tails by venipuncture to determine the fasting blood glucose (FBG).

Experimental mice were exposed to single intraperitoneal administration of freshly prepared alloxan monohydrate in normal saline at a dose of 170 mg/kg BW [Ma *et al.*, 2015; Ighodaro *et al.*, 2017]. Within 30 minutes of alloxan administration, the mice were allowed to feed on standard food pellets and water *ad libitum*. Thereafter, the mice were kept for 3 days (72 hours) [Tafesse *et al.*, 2017] and the blood glucose levels were determined. Alloxan treated mice with more than 75% increase in blood glucose levels using the formula below and/or with FBG of 11.1 mmol/l were considered diabetic hence selected for the study.

$$\%\ \text{increase in FBG} = \frac{FBG\ after\ alloxan - FBG\ before\ alloxan}{FBG\ before\ alloxan} \times 100$$

Whereby;

FBG before alloxan = Initial FBG recorded before alloxan administration

FBG after alloxan = FBG recorded after 72 hours of alloxan administration

2.9.4. Administration of extracts and hypoglycemic testing

Diabetic mice were intragastrically administered with test extract and fractions at the dose of 100 mg/kg BW once daily. Treatment commenced on third-day (72 hours) post induction of

diabetes considered as the first day of treatment and the study continued for 20 days. Blood glucose levels were recorded on day 1, 5, 10, 15 and 20 days of treatment.

Diabetic mice were randomly divided into groups each with 8 mice and treated as follows;
Group 1: Diabetic negative control - Diabetic mice administered with 5% ethanol 10 ml/kg BW
Group 2: Diabetic mice administered with the crude ethanolic extract at the dose of 100 mg/kg BW.
Group 3: Diabetic mice administered with the methanol fraction at the dose of 100 mg/kg BW.
Group 4: Diabetic positive control - Diabetic mice administered with chlorpropamide 100 mg/kg BW.

2.10. DETERMINATION OF ACUTE ORAL TOXICITY

Acute oral toxicity testing of the albino mice followed the procedures stipulated by the OECD guidelines 423 [OECD, 2001]. Mice were fasted with allowed access to water for 3-4 hours prior to the experiment.

Mice for determination of acute oral toxicity were grouped to involve each group with 4 mice, started with the dose of 2000 mg/kg BW abiding with procedures from the OECD guideline 423, Annexure 2c [OECD, 2001], which recommended that the starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The same procedure was followed for vehicle treated control group.

A single dose of extract was given and observed for 14 days for their behavioral (tremors, convulsions, salivation, diarrhoea, lethargy, sleep); respiratory changes as well as mortality.

Both groups were observed closely for any toxic effect within first 6 hours and then at regular intervals for a total period of 14 days. Weights of mice were monitored and at the end of study, mice were weighed. Vital organs were excised after killing mice by chloroform

inhalation, weight of organs was noted, and organ to body weight index calculated and then organs preserved in 40% formalin and sent for histopathological evaluation.

The formula used to calculate percentage organ to body weight index was;

$$organ\ to\ body\ weight\ index\ (\%) = \frac{organ\ weight\ (g)}{body\ weight\ (g)} \times 100$$

2.11. HISTOPATHOLOGICAL EVALUATION

Mice were humanely sacrificed and the vital organs (kidneys, lungs, hearts, livers, spleens and intestines) isolated, weighed and examined for macroscopic changes and/or development of any lesions [Dammeyer *et al.*, 2009], then stored in 40% formalin. Features of both treated and control groups were compared and then histologically evaluated by the Department of Pathology - MUHAS.

Tissues from mice were fixed for 24 hours in neutral well-buffered (40%) formalin, embedded in paraffin and sections (5 μm) mounted on SuperFrost slides (Menzel GmbH & CoKG, Braunschweig, Germany). These were then deparaffinized, rehydrated and stained with haematoxylin and eosin (H&E). Histological evaluation and photomicrography was performed by the Histopathologist using an Olympus (CX31RBSF Model) light microscope equipped with a digital camera (Olympus Corporation, Tokyo, Japan). Tissue toxicity (damage) was evaluated under the microscope on 7 low-power fields (x10 magnification) as well as on their high-power fields (x40 magnification) while taking pictures [Mwakigonja *et al.*, 2007; Dammeyer *et al.*, 2009]. Picture processing and printing was performed using Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA, USA).

2.12. PHYTOCHEMICAL SCREENING

Various chemical tests were performed on the extract to identify the phytoconstituents using standard procedures [Harborne, 1973; Evans, 2009; Njoku and Obi, 2009] with slight modifications. Each phytochemical test involved the testing concentrations of 10 mg/ml of the ethanolic crude extract.

2.12.1. Test for tannins

The ethanolic extract (2 ml) was stirred with 2 ml of distilled water, and a few drops of ferric chloride ($FeCl_3$) solution were added. The greenish/bluish-black precipitate formed indicated the presence of tannins

2.12.2. Test for saponins

The ethanolic extract (5 ml) was vigorously shaken with 5 ml of distilled water in a test tube and warmed. The mixture was then left for observation on the production of the persistent or stable foams indicated the presence of saponins.

2.12.3. Test for flavonoids

Dilute sodium hydroxide solution (1 ml) was added to a solution of 2 ml of the extract in water. Production of yellow color indicated the positive test or presence of flavonoids.

2.12.4. Test for terpenoids

The ethanolic extract (2 ml) was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 minutes. A greyish colour was formed indicated the presence of terpenoids.

2.12.5. Test for steroids

To ethanolic extract (2 ml), 2 ml of chloroform and 2 ml concentrated sulphuric acid were added; the red colour produced in the lower chloroform layer indicated the presence of steroids.

2.12.6. Test for alkaloids

Ethanolic extract (3 ml) was dissolved in 3 ml of dilute hydrochloric acid and filtered. Then, 2 ml of filtrate of plant drug extract was mixed with 2 ml of the Dragendorff's reagent. The reddish-brown precipitate formed indicated the presence of alkaloids.

2.12.7. Tests for cardiac glycosides

The extract (3 ml) was treated with 2 ml of acetic acid containing one drop of ferric chloride solution. Then, 1 ml of concentrated sulphuric acid was added. A brown ring formed at the interface indicated characteristic features of cardiac glycosides.

2.12.7.1. Tests for cardiac glycosides – aglycone

To the crude extract (3 ml), few drops of 1% picric acid solution and 10% sodium hydroxide solution were added, the colour of the extract retained to indicate the absence of characteristic aglycones.

2.13. STATISTICAL ANALYSIS

Results expressed as mean ± SD, and the level of statistical significance was determined using independent student t-test, when the difference between the means of two population groups was considered (each against the negative control). $p < 0.05$ was considered as a significant level.

2.14. ETHICAL CLEARANCE

The ethical clearance to conduct this study was granted from the Institutional Review Board, MUHAS, with Ref no. DA.287/298/01A/, and the use of mice followed internationally accepted principles for laboratory animal use and care stipulated in EEC Directive of 1986 [EEC, 1986]. Mice were housed in metal cages, with enough supply of air, and lighting sequence being 12 hours light, 12 hours dark in 24 hours, with the allowed access to food and water *ad libitum*.

The mice were humanely killed by inhalation of chloroform abiding to the international accepted principles for laboratory care and use of animals [CPCSEA, 2003] and disposed at MNH incinerator.

CHAPTER THREE

3. RESULTS AND DISCUSSIONS

3.1. EXTRACT AND FRACTION YIELDS

The total yield of the extraction was 4.4% (35 g). Amount of fractions and their corresponding percentages are presented in **Table 1**.

Table 1: The Percentage Yield of the Fractions of *S. terminale* Fruits from 20 g of the Crude Extract

Fraction	Mass Obtained (g)	% Yield
DCM	1.36	6.80
EtOAc	1.79	8.95
MeOH	11.44	57.20
H_2O	1.87	9.35

Key: DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, H_2O = water.

The yield of the fractions ranged from 6.80% to 57.20%. It appears that the fraction yield increased with increasing polarity of the solvent. The methanolic fraction yielded higher percent of all the fractions, followed by water, ethyl acetate and dichloromethane fractions respectively. The observation suggests that the nature of the major phytochemicals extracts present in *S. terminale* fruits were of high polarity.

The solvent polarity plays an important role in extraction and fractionation yields however in some studies [Markom *et al.*, 2007] the trend has been different. Water being more polar than methanol it was expected its fraction to be higher. However, in our study the reverse was observed. Similar trend has been reported before [Pin *et al.*, 2010]. This results could be due to various factors such as extraction temperature, extraction time and solvent to solid ratio that

my lead to variation of yields [Ajani *et al.*, 2017]. After fractionation the loss on weight was found to be 3.54 g.

3.2. OGTT MODEL

Following the oral glucose load, the mean blood glucose levels of the tested mice varied periodically (**Table 2**). Administration of the test extracts started 30 minutes before oral glucose loading in OGTT to enhance maximum absorption of the extract.

The overall blood glucose levels for all groups increased 30 minutes after glucose load, this is due to the physiologically induced hyperglycaemia caused by the raised blood glucose levels temporarily without damaging the pancreas [Singh and Pathak, 2015]. Then, the gradual decrease of the mean blood glucose levels was observed in the subsequent hours.

The crude ethanolic extract and different fractions of *S. terminale* fruits were administered to different treatment groups at 100 mg/kg BW and diverse responses towards blood glucose lowering effect were observed. After 1 hour of glucose oral load, ethyl acetate fraction reduced the blood glucose level close to the response of the positive control, while the crude extract and other fractions reduced the blood glucose levels in moderate values as presented in **Table 2**.

During the second hour, crude ethanolic extract and methanolic fraction had lowest blood glucose levels, while on the third hour of observation; the blood glucose levels for all groups were dropping at a fairly constant rate with just slight variations. Significant reduction of blood glucose levels after an initial rise in the first 30 minutes was observed. This results indicated the efficacy of both crude extract and fractions to lower blood glucose lowering [Chandran *et al.*, 2017].

This observation indicates the ability of the extract and fractions to provide tolerance to the glucose loaded, acting probably through stimulation of insulin release from the β-cells of the pancreas, acting in the same fashion as chlorpropamide, a sulfonylureas class of antidiabetic medication [Inzucchi, 2002].

The crude ethanolic extract and fractions significantly ($p < 0.05$) inhibited the increase of fasting blood glucose levels at different time intervals. Efficacy of the crude ethanolic extract on blood glucose lowering was nearly equal to that of the positive control. The inhibitory efficacy of the extract and fractions was comparable against the negative control testing group, where by the methanolic fraction had nearly twice the efficacy of the crude ethanolic extract on the overall statistics (see **Table 2**).

Several previous studies using animal models had demonstrated hypoglycaemic efficacy of DCM, ethyl acetate, aqueous and methanolic extracts and fractions from various medicinal plants and this is due to the presence of various bioactive molecules having an ability to reduce the blood glucose levels [Akah *et al.*, 2011; Chandran *et al.*, 2017; Erukainure *et al.*, 2018]. For example, *Solanum torvum* methanolic fruit extract reduced the blood glucose levels in streptozotocin induced diabetic rats at 200 and 400 mg/kg BW. At 300 mg/kg BW the methanolic leaf extract of *Solanum pubescens* reported to lower the elevated blood glucose and lipid parameters in alloxan induced diabetic rats [Kandimalla *et al.*, 2016]. The effect is associated with the efficacy of the plant to enhance insulin secretion probably due to regeneration of β-cells, reduce oxidative stress and modulate enzymes responsible for glucose metabolism [Gandhi *et al.*, 2011]. Therefore, the hypoglycaemic efficacy demonstrated by *S. terminale* fruits extract and fractions in OGTT model could be associated with any of the mechanisms as pointed out.

Table 2: Mean Blood Glucose levels for the OGTT

Time (Hours)	Mean Blood Glucose Levels (mmol/l) ± S.D, n = 5						
	Controls		Extract and Fractions at a dose 100 mg/kg body weight				
	Negative	Positive	Crude	DCM	EtOAc	MeOH	H_2O
0	3.6 ± 0.5	3.6 ± 0.3	3.2 ± 0.7	2.5 ± 0.5	3.9 ± 0.3	3.0 ± 0.1	2.5 ± 0.6
0.5	4.7 ± 0.5	3.9 ± 0.4	4.3 ± 0.5	4.6 ± 0.3	6.2 ± 0.5	4.1 ± 0.5	5.1 ± 0.4
1	4.4 ± 0.7	2.2 ± 0.1*	3.2 ± 0.6*	3.1 ± 0.2	2.7 ± 0.7*	3.4 ± 0.3*	3.1 ± 0.3*
2	4.2 ± 0.5	3.1 ± 0.2*	2.9 ± 0.7*	3.2 ± 0.4*	3.1 ± 0.4*	2.8 ± 0.5*	3.2 ± 0.3*
3	4.0 ± 0.7	3.2 ± 0.4*	3.1 ± 0.3*	3.4 ± 0.4	3.1 ± 0.3*	3.2 ± 0.6*	3.5 ± 0.1

Key: **Negative Control** - 5% Ethanol; **Positive Control** – 100 mg/kg BW chlorpropamide

* indicates the statistical significance, *p*-value < 0.05 with the negative control by student's t - test

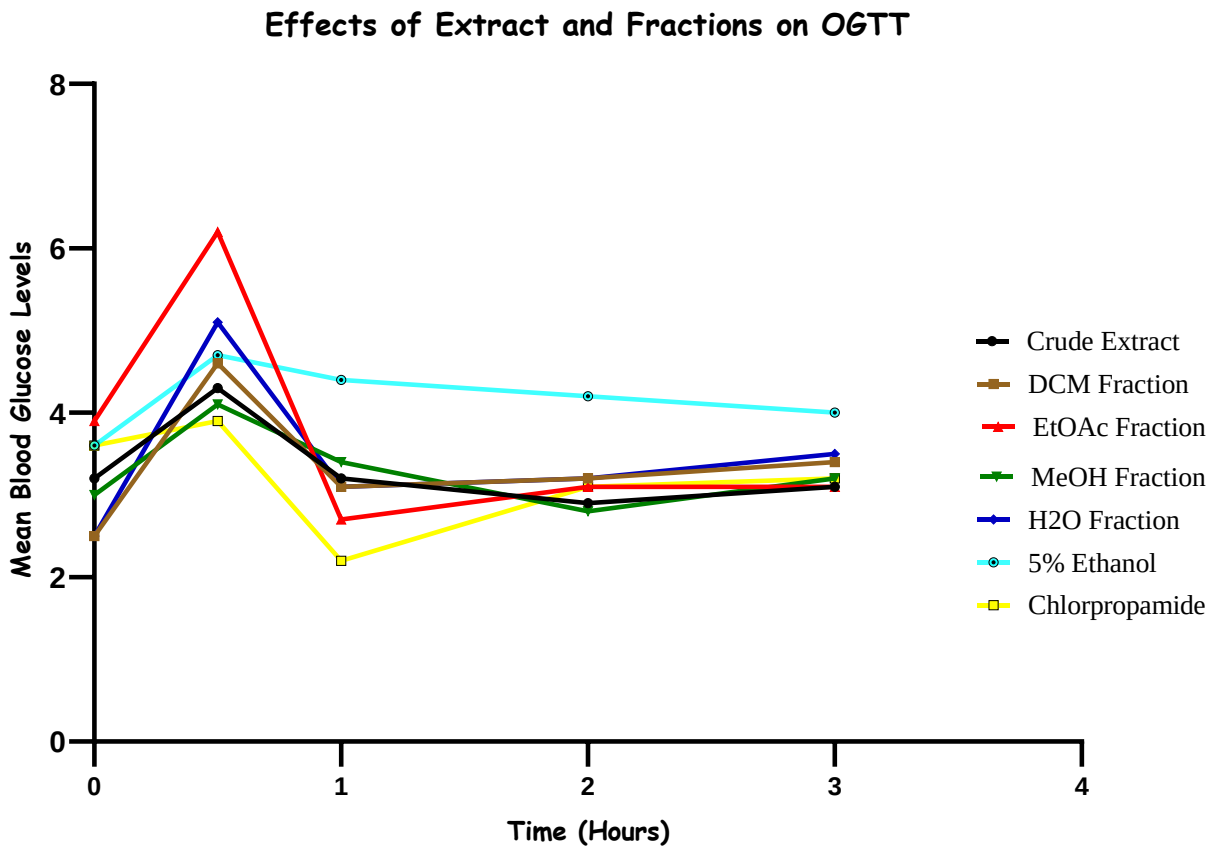

Figure 6: Effects of Extract and Fractions on OGTT

3.3. ALLOXAN MODEL

Alloxan induced diabetic mice treated with crude ethanolic extract and methanolic fraction of *S. terminale* fruits, once daily orally for 20 days demonstrated varying antidiabetic efficacy at a dose of 100 mg/kg BW.

Day 1 which was 72 hours after alloxanization of mice to induce diabetes was observed with the elevated FBG levels. This elevated FBG levels observed were in line with other symptoms of diabetes including increasing rate of urination (polyuria), increasing rate of water drinking associated with much thirsty (polydipsia), increased amount of food consumption (polyphagia) and weakness.

The overall trend of the mean FBG levels for the whole treatment period as presented in the **Table 3**. The mean blood glucose levels of the crude extract, methanolic fraction and chlorpropamide treated diabetic mice decreased from day 5 of treatment in contrast to the 5% ethanol treated diabetic mice. After 20 days, the reduction of the mean blood glucose levels for all groups were chlorpropamide > crude extract > methanolic fraction > 5% ethanol (see **Figure 7**). At a dose of 100 mg/kg BW, chlorpropamide managed to reduce 70% of the increased FBGs, where by the *S. terminale* ethanolic crude extract reduced nearly 60% and the methanolic fraction by 53% (see **Figure 8**), statistically only chlorpropamide and crude ethanolic extract managed to significantly lower the FBG ($p < 0.05$) from day 5. Some studies, for example, Moshi *et al.,* (1997) reported the ability of the *Phyllanthus amarus* extracts to clear the glucose load however, they did not significantly lower the FBG [Moshi *et al.*, 1997].

The observed weight of mice before and after alloxanization indicated less decrease percentage in weight on methanolic fraction and crude extract compared to the percentage in weight reduction on the 5% ethanol and chlorpropamide treated (**Table 4**). The animals slightly did not lose body weight with the crude extract and methanolic fraction, as compared with the vehicle-treated group, which signifies the valuable effect of the fruits in preventing further loss of body weight as seen with an aqueous extract of *Pleurotus pulmonarius* [Badole *et al.*, 2006].

In our study, *S. terminale* fruits crude extract and fractions had shown both statistical and clinical significance of lowering blood glucose levels in OGTT and alloxan models in mice, which signifies the efficacy of the plant in the management of diabetes. These results were similar to various plants. For instance; different extracts and fractions of *Solanum xanthocarpum* [Gupta *et al.*, 2009; Poongothai *et al.*, 2011], *Croton zambesicus* [Okokon *et al.*, 2011], and *Indigofera pulchra* [Tanko *et al.*, 2011].

The results being statistically significant does not give an assurance that the results are clinically relevant on a particular disease. Despite proving misleading results in some clinical environment, statistical significance testing rarely determines the practical importance or clinical relevance of some findings [Armijo-Olivo *et al.*, 2011]. In some instances, consideration on clinical relevance is important which signifies if the study results are meaningful clinically.

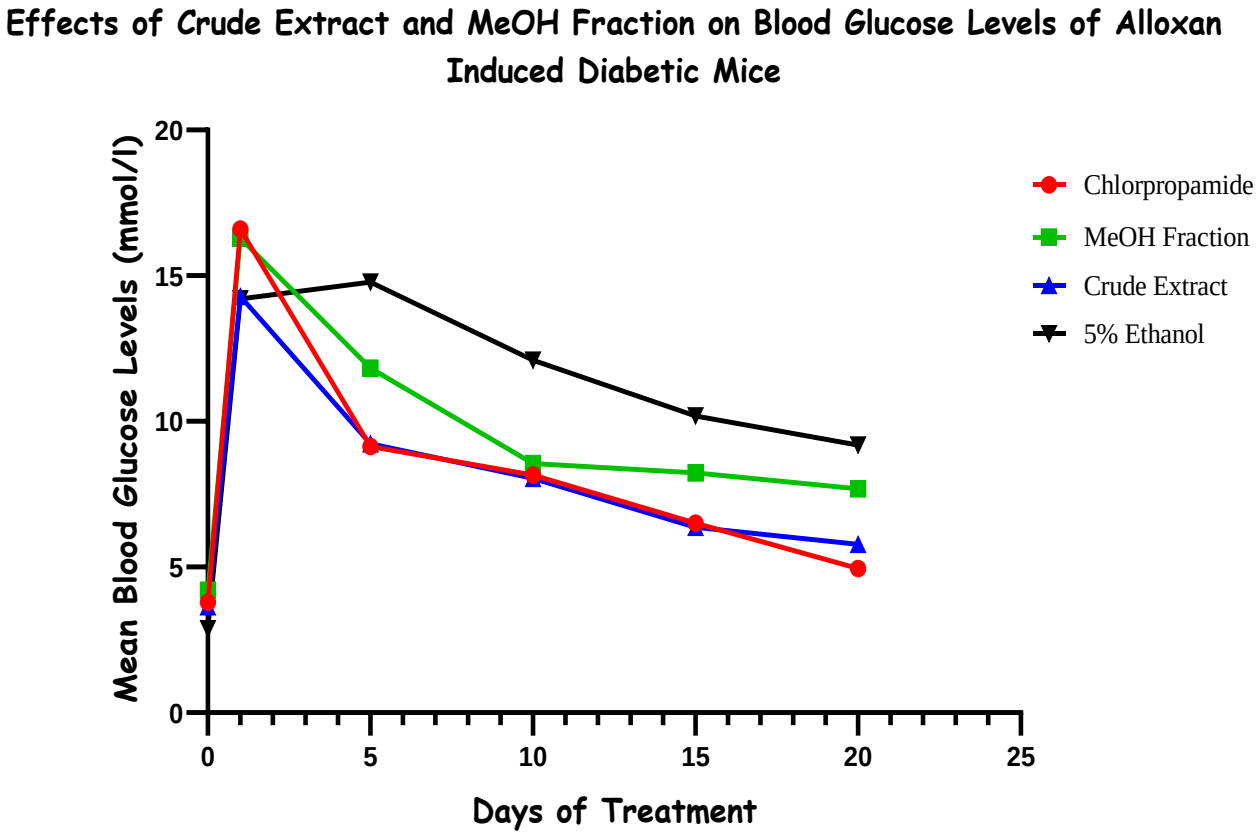

Figure 7: Effects of Extract and Fraction of *S. Terminale* Fruits in Alloxan Induced Diabetic Mice

Table 3: Mean FBG (mmol/l) of Alloxan Treated Diabetic Mice

Time (Days)	Mean Blood Glucose Levels (mmol/l)± S.D, n = 8			
	Controls		Extract and Fractions at a dose 100mg/kg body weight	
	Negative	Positive	Crude Extract	MeOH Fraction
0	2.88 ± 0.84	3.79 ± 1.63	3.64 ± 1.17	4.2 ± 1.34
1	14.20 ± 5.53	16.6 ± 3.99	14.29 ± 2.35	16.29 ± 3.09
5	14.78 ± 7.42	9.14 ± 4.51*	9.23 ± 2.89*	11.83 ± 5.02
10	12.10 ± 6.96	8.16 ± 3.51*	8.04 ± 2.52*	8.55 ± 3.22*
15	10.18 ± 4.40	6.5 ± 4.34*	6.36 ± 2.58*	8.23 ± 3.44*
20	9.18 ± 4.38	4.95 ± 3.26	5.78 ± 2.65	7.68 ± 3.40

* indicates the statistical significance, *p*-value < 0.05 with the negative control by student's t -test

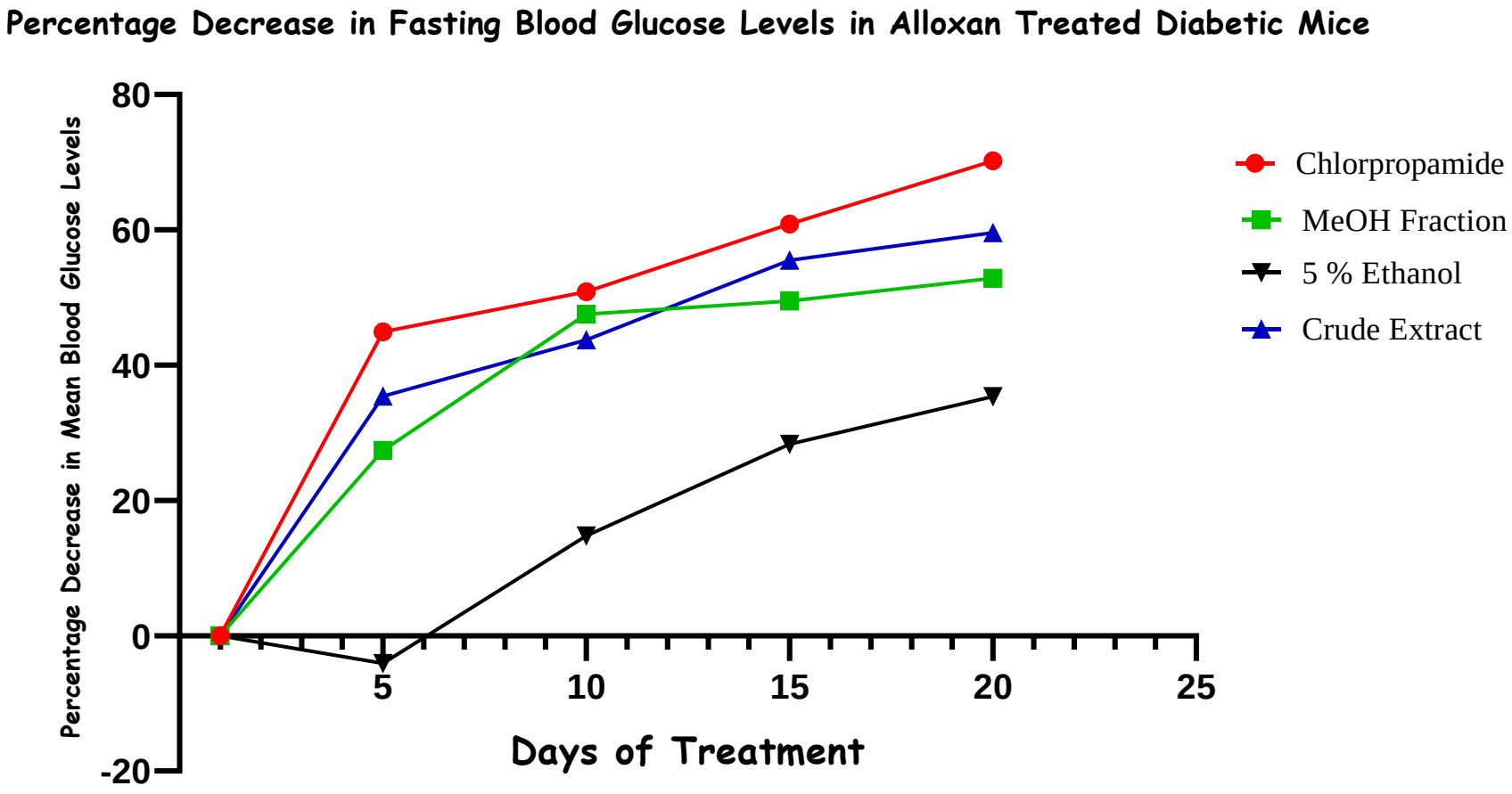

Figure 8: Percentage Decrease in Fasting Blood Glucose Levels in Alloxan Treated Diabetic Mice

Table 4: Mean Body Weights of Alloxan Treated Mice

Groups	Mean body weights ± S.D (g), n = 8						% weight decrease
	Day 0	Day 1	Day 5	Day 10	Day 15	Day 20	
Control – 5% Ethanol	27.63 ± 3.78	26.25 ± 4.03	24.75 ± 3.73	24.25 ± 4.27	24.13 ± 5.06	23.00 ± 5.68	16.75⸸ 12.38‡
Crude Extract 100mg/kg BW	27.25 ± 1.83	27.13 ± 2.30	26.00 ± 2.62	26.38 ± 2.67	25.88 ± 3.52	25.38 ± 3.85	6.86⸸ 6.45‡
Methanolic Fraction 100mg/kg BW	27.25 ± 2.43	26.63 ± 2.92	25.75 ± 3.96	26.63 ± 3.96	26.38 ± 4.10	25.75 ± 3.92	5.50⸸ 3.30‡
Chlorpropamide 100mg/kg BW	24.63 ± 3.07	23.88 ± 3.94	22.63 ± 4.44	23.00 ± 4.47	23.38 ± 4.34	22.25 ± 3.58	9.66⸸ 6.82‡

Key: ⸸% in weight before alloxan administration (day 0); ‡ % in weight after alloxan administration (day 1)

3.4. ACUTE ORAL TOXICITY

Medicinal plants contribute significantly in the drug discovery and development, as they have been used long ago for management of various minor and chronic ailments. To qualify being a drug candidate, the medicinal plant should possess no potential toxicity profile or should have low toxicity index, even after a long term use [Saleem *et al.*, 2017]. Assessment of toxicity profile of a medicinal plant is very essential, as majority of people in the society believe that all natural products especially plants are safe [Pariyani *et al.*, 2015]. Contrary to that notion, several plants used for management of diverse health conditions have been reported to demonstrate the toxic effects [Saleem *et al.*, 2017]. Moreover, irrespective of the prominent use of medicinal plants especially in traditional medicine, safety and efficacy have not been established for majority of these plants [Ng'uni *et al.*, 2018].

This fact demanded to assess the acute oral toxicity of the *S. terminale* ethanolic fruit extract in this study, which might result within a short time period following a single oral administration.

3.4.1. General Observations

No mortality was observed at the oral dose of 2000 mg/kg BW of the crude ethanolic fruit extract of the plant to all mice exposed, as well as there was no abnormal change in behavioral characteristics of mice during all 14 days of observation. The summary of overall observations is presented in **Table 5**. Organs morphology was normal in both control and treated groups (see **Figure 9**).

This result gives the indication that, the LD_{50} of *S. terminale* ethanolic fruit extract is estimated to be above 2000 mg/kg BW, which might be accepted to be safe according to the guidelines [OECD, 2001]. Several plants have been reported to have LD_{50}, within the ranges of our results such as, Dahanukar *et al*., (2000) reported and cited the LD_{50} of *Vitex leucoxylon* ethanolic leaf extract to be above 3000 mg/kg BW. While there have been reports of LD_{50} of extracts such as *Vitex leucoxylon* cold water infusion extract to be 1050 mg/kg BW on rats, *Ailanthus excelsa* ethanolic extracts at 1000 mg/kg BW, *Toddalia asiatica* at 350

mg/kg BW and 250 mg/kg BW for *Araucaria bidwilli* [Dahanukar *et al.*, 2000] which were lower than our extracts.

The Organization for Economic Co-operation and Development (OECD) recommended the chemical labelling and classification of acute systemic toxicity of extracts and substances based on oral LD_{50} values being: very toxic < 5 mg/kg BW, toxic > 5 < 50 mg/kg BW, harmful, > 50 < 500 mg/kg BW, and no label > 500 < 2000 mg/kg BW [Walum, 1998].

With this classification based on oral LD_{50} values, *S. terminale* ethanolic fruit extract at 2000 mg/kg BW could be regarded as safe for use. But this requires further safety studies to confirm for their safety.

Table 5: Behavioral Patterns for Acute Oral Toxicity - Control and Treated Mice

Parameters	Observations											
	30 Minutes		4 Hours		24 Hours		48 Hours		7 Days		14 Days	
	T	C	T	C	T	C	T	C	T	C	T	C
Tremors	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O
Convulsions	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O	N-O

Key: C = Control Group 5% Ethanol; T = Treated Group with STE; N-O = Not observed, N = normal

Control – 5 % Ethanol

Treated Mice – 2000 mg/kg BW

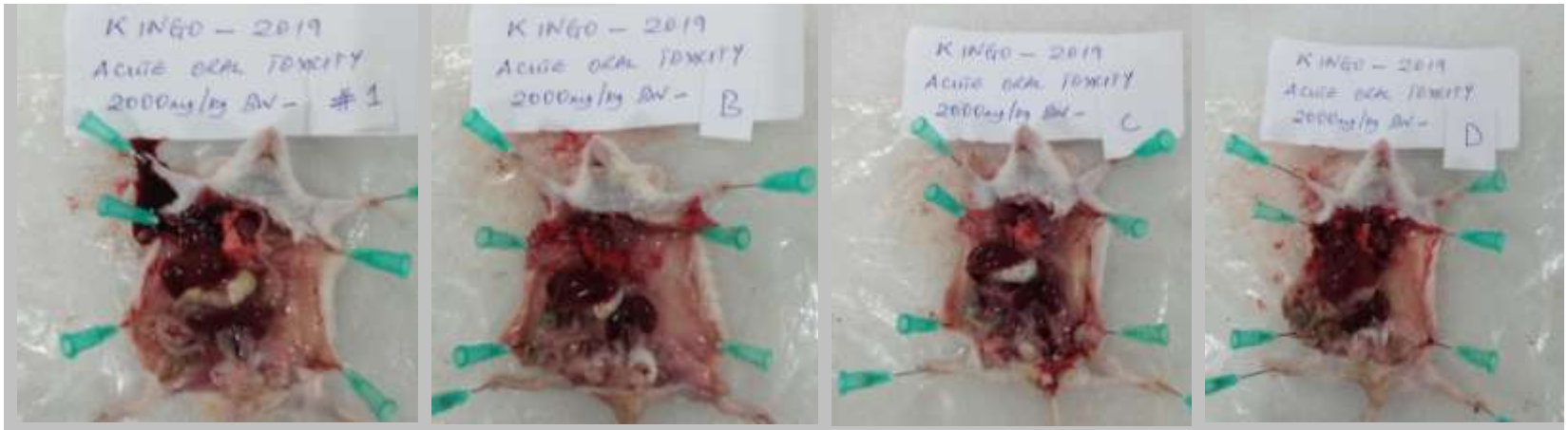

Figure 9: Morphological Observations for Acute Oral Toxicity in Mice at 2000 mg/kg BW

3.4.2. Body Weight

There were no significant variations observed in body weights of both treated and control mice groups in acute toxicity testing of the crude ethanolic extract. This indicates the possibility of the fruits not interfering with the feeding pattern of the mice.

Table 6: Effects of Extract in Body Weight of Mice in Acute Toxicity Testing

Groups	Mean body weights ± S.D (g), n = 4		
	Day 1	Day 7	Day 14
Control – 5% Ethanol	27.4 ± 1.1	30.8 ± 1.8	30.8 ± 1.6
Crude Extract 2000mg/kg BW	28.0 ± 1.4	32.2 ± 1.6	31.8 ± 1.1

3.4.3. Organs Weight

Liver, kidney, intestine, heart, spleen and lung isolated from mice of both groups involved in the acute oral toxicity testing, were observed with no any abnormal lesions. There were no significant variations in the measured mean organ weights observed as presented in **Table 7** and the percentage of organ to body weight index was minimal.

Organ to body weight index (also known as the relative organ weight index) expressed in percentage or fraction is one of significant indicators in assessing potential harmful effects of plant extracts and fractions. The toxicity of extracts, fractions or any substance on the internal organs could be identified by assessing the relative organ weight as the index, which gives a preliminary insight to the swelling or damage caused by any harmful agent [Pariyani *et al.*, 2015]. Moreover, it has been considered that relative weights indices of internal organs reveal the extent of metabolic burden on the organ, mainly by its basic function [Liro, 1985]. With the observed low organ body weight index variations (**Table 7**), indicates that the extract at 2000 mg/kg BW is relatively less toxic. When body weight variations is above 10%, indicates potential toxicity of the extract/fraction [Vaghasiya *et al.*, 2011].

Table 7: Mean Organ Weights

Organs	Mean organ weights ± S.D (g)		Organ to body weight index (%)		% Increase or Decrease
	2000 mg/kg BW	5% Ethanol	2000 mg/kg BW	5% Ethanol	
Liver	2.04 ± 0.36	1.70 ± 0.5	6.42	5.51	+0.91↑
Heart	0.14 ± 0.01	0.16 ± 0.05	0.45	0.52	-0.07↓
Kidney	0.55 ± 0.07	0.54 ± 0.06	1.72	1.75	-0.03↓
Intestines	2.54 ± 0.37	2.55 ± 0.43	7.97	8.28	-0.31↓
Spleen	0.28 ± 0.12	0.20 ± 0.12	0.88	0.66	+0.22↑
Lungs	0.22 ± 0.02	0.24 ± 0.05	0.70	0.78	-0.08↓

Key: ↓ = % decrease, ↑ = % increase, S.D = standard deviation, n = 4

3.5. HISTOPATHOLOGICAL EVALUATIONS

The histopathological analysis revealed the normal organ structures in control animals except for lungs where mild to moderate focal pneumonic infiltrates were observed as presented in **Figure 10**. These infiltrates could be possibly caused by environment in which animals were kept before and during the experiment since they were found in both control and treated groups, and thus remains as the confounding factor. Also, slight myocardial muscle degeneration was observed in the heart of treated mice groups as shown in **Figure 11**, indicating signs of mild cardiotoxicity. Stomach and intestines were normal, indicating no structure disorganization in the gastrointestinal tract after extract oral administration, as presented in **Figure 12**.

Mild congestion in capillary glomerulus and thrombosis was seen in the kidney of the treated mice groups as shown in **Figure 13**; persistence of this condition is life threatening that may lead to kidney injury and other clinical manifestations [Navarro *et al.*, 2017]. Mild focal

inflammatory reactions of the liver were also observed in the treated mice shown in **Figure 14** as steatosis which occurs due to toxic stress [Koyama and Brenner, 2017].

Red pulp hyperplasia of the spleen was observed in the treated mice as presented in **Figure 15** which is described as malformation since spleen is the primary target indicating the direct and indirect toxicity of various agents [Suttie, 2006]. Therefore, loss of the intact organization in the spleen could be associated with administered *S. terminale* crude fruits ethanolic extract demonstrating potential toxicity effect of the extract at higher doses.

The plant may still be considered suitable for further research since antidiabetic activity was observed at 100 mg/kg BW regardless of the sign of mild cardiotoxicity and nephrotoxicity observed at 2000 mg/kg BW of *S. terminale* crude fruits ethanolic dose tested for acute oral toxicity. This is obvious, since traditionally people consume few fruits per day.

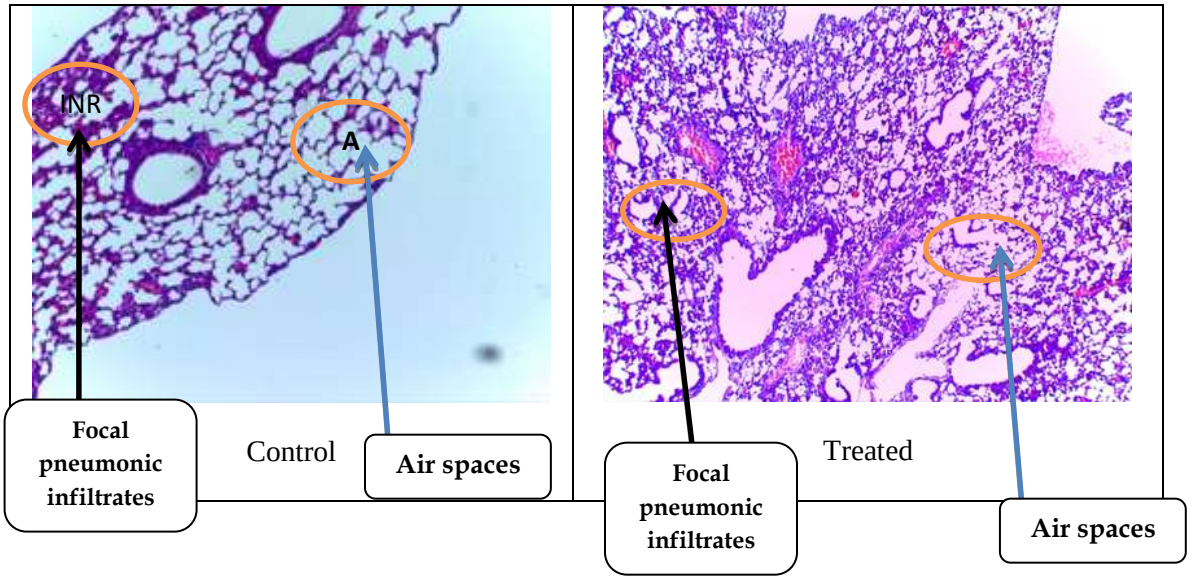

Figure 10: Photomicrographs of the Lungs

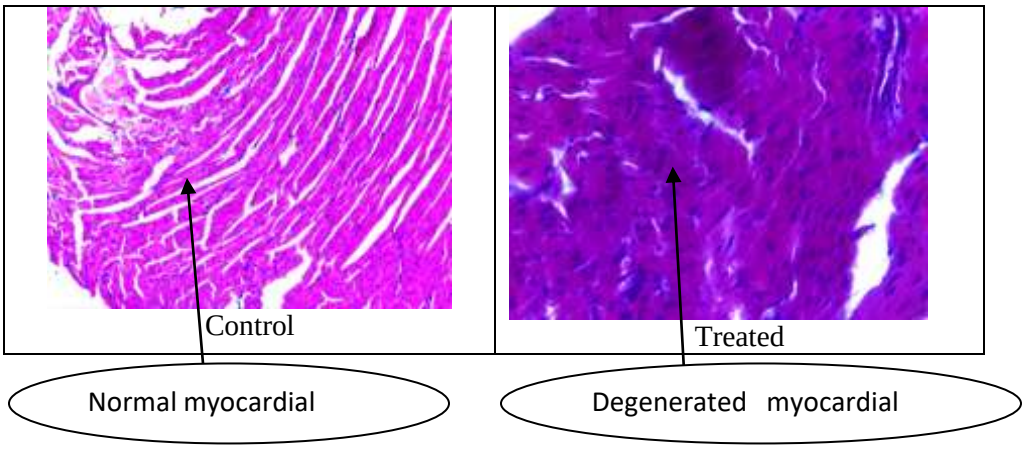

Figure 11: Photomicrographs of the Heart

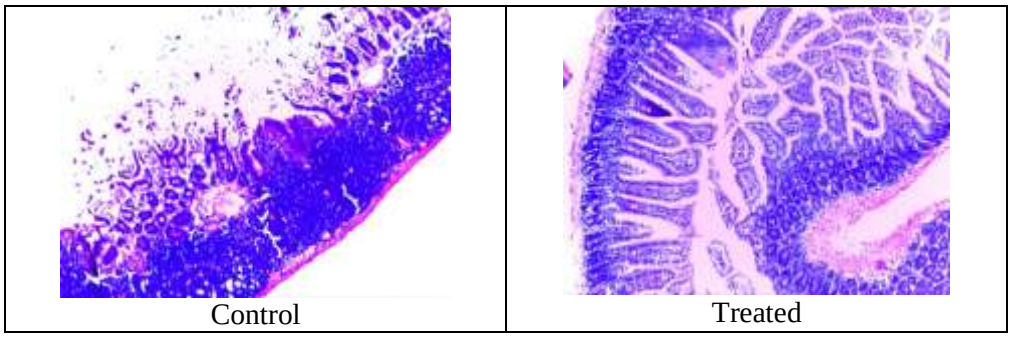

Figure 12: Photomicrographs of the Gastro intestines

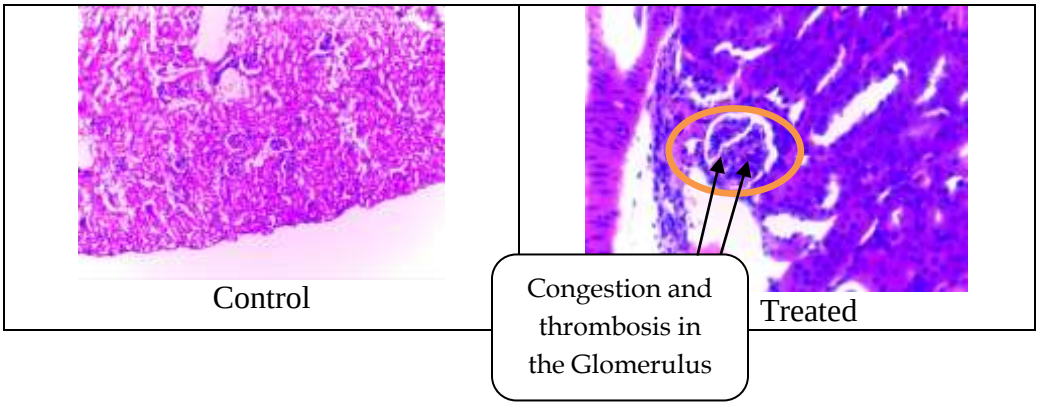

Figure 13: Photomicrographs of the Kidney

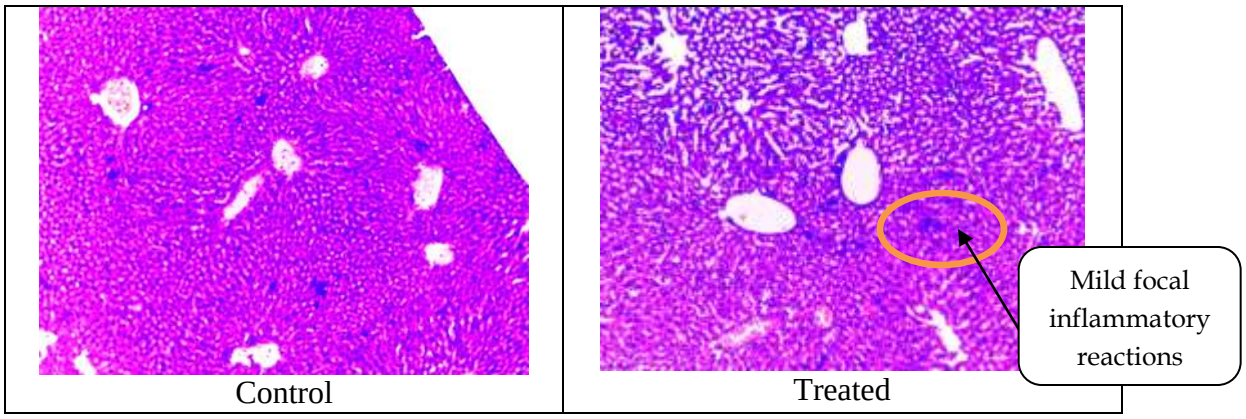

Figure 14: Photomicrographs of the Liver

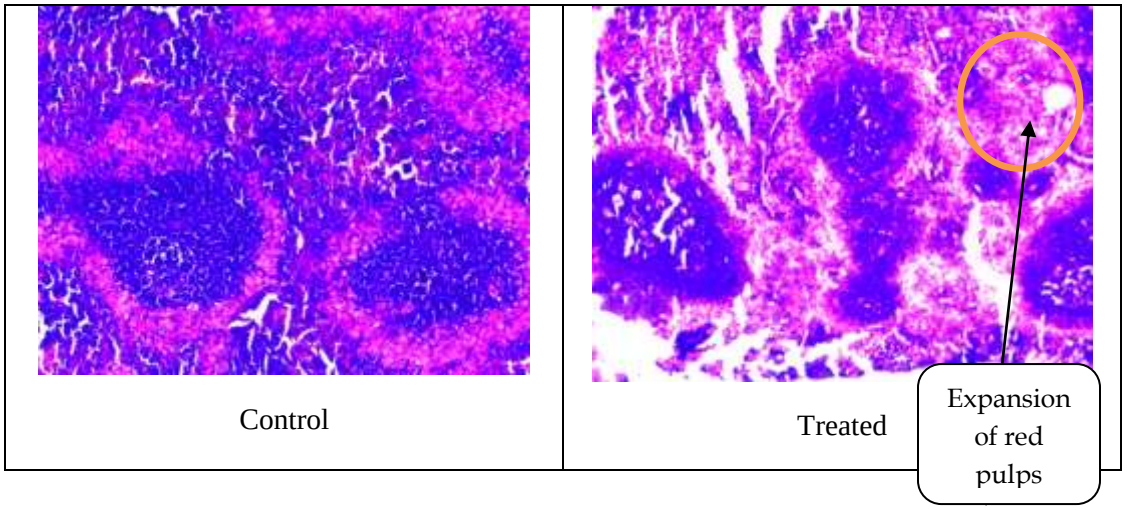

Figure 15: Photomicrographs of the Spleen

3.6. PHYTOCHEMICAL SCREENING

Phytochemical screening results for the crude ethanolic extract of *S. terminale* fruits, demonstrated the presence of seven phytochemical groups as presented in **Table 8**.

Table 8: Phytochemical Groups of *S. Terminale* Crude Ethanolic Fruit Extract

Phytochemical Group	Chemical test	Inference
Alkaloids	Dragendorff's test	+
Flavonoids	Dilute NaOH test	+
Tannins	Ferric Chloride test	+
Steroids	Salkowski Test	+
Saponins	Foam test	+
Cardiac Glycosides	Keller – Kilian test	+
Cardiac Glycosides (aglycone)	Baljet test	-
Terpenoids	Salkowski Test (modified)	+

Key, **(+) present, (-) absent**

From the literature, alkaloids, carbohydrates, resins, saponins, flavonoids, proteins, coumarins, anthraquinones, terpenoids, minerals, phenolics, and steroids from various plants have been reported to exhibit hypoglycaemic effect [Gaikwad *et al.*, 2014] through different mechanisms of action including insulin like action or secretion, regeneration of pancreatic β-cells, protective effect on hepato-pancreatic system, reduced glucose absorption, favouring peripheral glucose utilization, inhibition glycogen-metabolizing enzymes, etc. [Bharti *et al.*, 2018]. Of these phytochemicals, saponins are well known to possess antidiabetic effect and

are the main promising compounds potentially to be developed into new antidiabetic medications [El *et al.*, 2017].

Similarly, alkaloids, glycosides, carbohydrate, and bitter principles have also been associated in the antidiabetic activities of many plants [Slijepcevié, 1991].

The crude ethanolic fruit extract of *S. terminale* showed ranges of phytochemicals (**Table 8**), giving the maximum potential of the fruits against diabetes. Further phytochemical quantitative analysis should be conducted to quantify amount of each chemical class of compounds, followed by isolation of the most active compounds and structure elucidation.

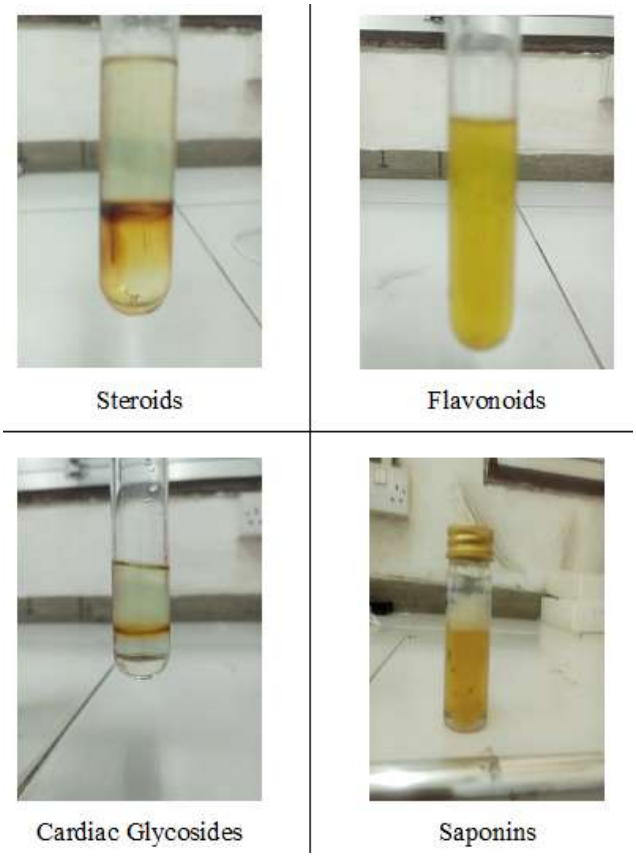

Figure 16: Some of the Colour Reactions for the Preliminary Phytochemical Screening Results

CHAPTER FOUR

4. CONCLUSION AND RECOMMENDATIONS

4.1. CONCLUSIONS

This study supports the claim of the traditional use of *S. terminale* fruits in diabetes management in Lushoto district of Tanzania. The crude ethanolic extract and fractions showed hypoglycaemic activity in OGTT and Alloxan models suggesting their possession of antidiabetic actions. Based on our results, the ethanolic extract gave the best results suggesting high probability of polar and less polar compounds working in a synergistic manner in the reduction of blood glucose levels.

Since *S. terminale* has antidiabetic effect, precautions should be taken when administered for other diseases among non-diabetics, as traditionally people do use these fruits for the management of hypertension as well.

Meanwhile the observed antidiabetic dose of the crude ethanolic extract, i.e. 100 mg/kg BW, is far much low compared to 2000 mg/kg BW dose tested for toxicity in mice. At this 2000 mg/kg BW, *S. terminale* crude extract caused no behavioral changes but caused mild organ damage. This plant qualifies for further work in search of antidiabetic compounds/standardization of herbal medicine. However, assurance on safety of *S. terminale* fruits is yet to be determined after performing other toxicity studies using different models and parameters.

Furthermore, the various phytochemicals (alkaloids, flavonoids, tannins, steroids, saponins cardiac glycosides and terpenoids) present in the crude ethanolic fruit extract of *S. terminale* had previously been reported for their efficacy in lowering blood glucose levels exhibiting various mechanisms from other plants, suggesting the presence of potential compounds for antidiabetic activity.

4.2. RECOMMENDATIONS

Further work is needed including; (i) To carry out various *in vivo* and *in vitro* techniques to confirm the antidiabetic activities and mechanism of action (ii) To carry out more toxicological studies such as, sub chronic and chronic toxicity, genotoxicity, embryo toxicity, haematological toxicity etc., so as to ascertain the safety status of *S. terminale* fruits (iii) To identify and isolate the most active compounds (iv) To standardize and, (v) To formulate *S. terminale* fruits herbal products to facilitate availability even during the off season and ease of distribution to the consumers.

MUHAS animal house should be improved to avoid animal contamination.

4.3. LIMITATIONS

The results of this dissertation could be influenced by the following as limitations during conduction of the research, by which should be taken into considerations upon future similar studies.

i. Data collection and data collection tools.

Data collection tools i.e., glucometers and procedures of blood collecting and recording should be validated to avoid systematic errors.

ii. Sample size

Number of the animal subjects to be involved in the study should be justifiable and representative. Taking note of the 3R (Reduction, Replacement, and Refinement) ethical practice of animal use in research.

CHAPTER FIVE

5. REFERENCES

Adams, G. G., Imran, S., Wang, S., Mohammad, A., Kok, S., Gray, D. A., Channell, G. A., Morris, G. A., Harding, S. E. (2011) 'The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines', *Food Research International*. Elsevier Ltd, 44(4): 862–867.

Afolayan, A. J. and Sunmonu, T. O. (2010) 'In vivo Studies on Antidiabetic Plants Used in South African Herbal Medicine', *Journal of Clinical Biochemistry and Nutrition*, 47: 98–106.

Ajani, E. O., Sabiu, S., Odufuwa, K. T., Ibrahim, T. B., and Salau, B. A. (2017) 'Evaluation of Lens Aldose Reductase Inhibitory and Free Radical Scavenging Activity of Purified Fractions of Hyptis suaveolens Flower: Potential for Cataract Remediation', *Pharmacognosy Journal*, 9(1): 62–9.

Akah, P. A., Uzodinma, S. U. and Okolo, C. E. (2011) 'Antidiabetic activity of aqueous and methanol extract and fractions of gongronema latifolium (asclepidaceae) leaves in alloxan diabetic rats', *Journal of Applied Pharmaceutical Science*, 1(9): 99–102.

Almanca, C. C. J., Saldanha, S. V., Sousa, D. R., Trivilin, L. O., Nunes, L. C., Porfírio, L. C., and Marinho, B. G. (2011) 'Toxicological evaluation of acute and sub-chronic ingestion of hydroalcoholic extract of Solanum cernuum Vell. in mice', *Journal of Ethnopharmacology*, 138(2): 508–512.

American Diabetes Association (2014) 'Diagnosis and Classification of Diabetes Mellitus', *Diabetes Care*, 37(1): S81–S90.

Armijo-Olivo, S., Warren, S., Fuentes, J., and Magee, D. J. (2011) 'Clinical relevance vs. statistical significance: Using neck outcomes in patients with temporomandibular disorders as an example', *Manual Therapy*. Elsevier Ltd, 16(6): 563–572.

Auerbach, B. J., Reynolds, S. J., Lamorde, M., Merry, C., Kukunda-Byobona, C., Ocama, P., Semeere, A. S., Ndyanabo, A., Boaz, I., Kiggundu, V., Nalugoda, F., Gray, R. H., Wawer, M.

J., Thomas, D. L., Kirk, G. D., Quinn, T. C., and Stabinski, L. (2012) 'Traditional Herbal Medicine Use Associated with Liver Fibrosis in Rural Rakai, Uganda', *PLoS ONE*, 7(11): 5–12.

Augustino, S., Hall, J. B., Makonda, F. B., and Ishengoma, R. C. (2011) 'Medicinal Resources of the Miombo woodlands of Urumwa, Tanzania: Plants and its uses', *Journal of Medicinal Plants Research*, 5(27): 6352–6372.

Badole, S. L., Shah, S. N., Patel, N. M., Thakurdesai, P. A., and Bodhankar, S. L. (2006) Hypoglycemic activity of aqueous extract of Pleurotus pulmonarius in alloxan-induced diabetic mice', *Pharmaceutical Biology*, 44(6): 421–425.

Balogun, F. O., Tshabalala, N. T. and Ashafa, A. O. T. (2016) 'Antidiabetic Medicinal Plants Used by the Basotho Tribe of Eastern Free State: A Review', *Journal of Diabetes Research*, 2016: 1–13.

Bedekar, A., Shah, K. and Koffas, M. (2010) *Natural products for type II diabetes treatment.* 1st edn, *Advances in applied microbiology*. 1st edn. Elsevier Inc.

Bharti, S. K., Krishnan, S., Kumar, A., and Kumar, A. *et al.* (2018) 'Antidiabetic phytoconstituents and their mode of action on metabolic pathways', *Therapeutic Advances in Endocrinology and Metabolism*, 9(3), pp. 81–100.

Braguini, W. L., Pires, N. V. and Alves, B. B. (2018) 'Phytochemical analysis, antioxidant properties and Brine Shrimp lethality of unripe fruits of Solanum viarum', *Journal of Young Pharmacists*, 10(2): 159–163.

Chandran, R., Parimelazhagan, T. and George, B. P. (2017) ' Antihyperglycemic activity of the bark methanolic extract of Syzygium mundagam in diabetic rats ', *Alexandria Journal of Medicine*. Alexandria University Faculty of Medicine, 53(4): 317–324.

Chang, C., Lin, Y., Bartolome, A. P., Chen, Y., Chiu, S., and Yang, W. (2013) 'Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology, and Potential Application of

Selected Plants and Compounds', *Evidence-based complementary and alternative medicine : eCAM*, 2013:1–33.

Chiwanga, F. S., Njelekela, M. A., Diamond, M. B., Bajunirwe, F., Guwatudde, D., Nankya-Mutyoba, J., Kalyesubula, R., Adebamowo, C., Ajayi, I., Reid, T. G., Volmink, J., Laurence, C., Adami, H., Holmes, M. D., and Dalal, S. (2016) 'Urban and rural prevalence of diabetes and pre- diabetes and risk factors associated with diabetes in Tanzania and Uganda', *Global Health Action*, 9716(February).

Cho, N. H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, A.W., Malanda, B. (2018) 'IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045', *Diabetes Research and Clinical Practice*. Elsevier B.V., 138:271–281.

Choi, C. W., Song S. B., Oh J. S., and Kim Y. H. (2015) 'Antiproliferation Effects of Selected Tanzania Plants', *African Journal of Traditional, Complementary and Alternative Medicines*, 12(2): 96–102.

Choi, W. C. and Roh, S. G. (2011) 'Antidiabetic Synergistic Effects of Medicinal Plant Extract Mixtures on db/db Mice', *Journal of Life Science*, 21(2): 165–175.

CPCSEA (2003) 'CPCSEA Guidelines for Laboratory Animal Facility', *Indian Journal of Pharmacology*, 35(13): 257–274.

Credo, D., Machumi, F. and Masimba, P. J. (2018) 'Phytochemical Screening and Evaluation of Ant-diabetic Potential of Selected Medicinal Plants used Traditionally for Diabetes Management in Tanzania', *Internation Journal of Research in Pharmacy and Chemistry*, 8(3): 405–411.

Dahanukar, S. A., Kulkarni, R. A. and Rege, N. N. (2000) 'Pharmacology of medicinal plants and natural products.', *Indian Journal of Pharmacology*, 32: S81–S118.

Damian, D. J., Kimaro, K., Mselle, G., Kaaya, R., Lyaruu, I. (2017) 'Prevalence of

overweight and obesity among type 2 diabetic patients attending diabetes clinics in northern Tanzania', *BMC Research Notes*. BioMed Central, 10(1): 1–6.

Dammeyer, P., Mwakigonja, A. R., Rethi, B., Chiodi, F., and Wolpert, E. Z. (2009) 'Vaccination with β2-microglobulin-deficient dendritic cells protects against growth of β2-microglobulin-deficient tumours', *Scandinavian Journal of Immunology*, 70(1): 44–52.

Day, C. and Bailey, C. J. (2006) *Traditional Herbal Medicines for Modern Times - Antidiabetic Plants*. Edited by A. Soumyanath. New York: Taylor & Francis Group.

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., Hu, F. B., Kahn, C. R., Raz, I., Shulman, G. I., Simonson, D. C., Testa, M. A., Ram, W. (2015) 'Type 2 Diabetes Mellitus', *Nature Reviews*. Macmillan Publishers Limited, 1(July): 255–272.

Doss, A., Palaniswamy, M., Angayarkanni, J., and Dhanabalan, R. (2009) 'Antidiabetic activity of water extract of Solanum trilobatum (Linn.) in alloxan-induced diabetes in rats', *African Journal of Biotechnology*, 8(20): 5551–5553.

Drucker, D. J. and Nauck, M. A. (2006) 'The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes', *Lancet*, 368(9548):1696–1705.

Dsouza, D. and Lakshmidevi, N. (2015) 'Models to study in vitro antidiabetic activity of plants : A review', *International Journal of Pharma and Bio Sciences*, 6(3): 732–741.

EEC (1986) 'Council Directive 86/609 EEC for the protection of animals used for experimental and other scientific purposes', *Official Journal*, L358(L): 1–28.

El, B. A., Hussein, S. A., Alm-Eldeen, A., Hafez, A., and Mohamed, T. (2017) 'Diabetes Management Saponins and their potential role in diabetes mellitus', *Diabetes Manag*, 7(1): 148–158.

Elekofehinti, O. O., Kamdem, J. P., Kade, I. J., Rocha, J. B. T., Adanlawo, I. G. (2013)

‘Hypoglycemic, antiperoxidative and antihyperlipidemic effects of saponins from Solanum anguivi Lam. fruits in alloxan-induced diabetic rats’, *South African Journal of Botany*. Elsevier B.V., 88: 56–61.

Erasto, P., Grierson, D. S. and Afolayan, A. J. (2006) ‘Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina’, *Journal of Ethnopharmacology*, 106(1): 117–120..

Erukainure, O. L., Hafizur, R. M., Kabir, N., Choudhary, M. I., Atolani, O., Banerjee, P., Preissner, R., Chukwuma, C. I., Muhammad, A., Amonsou, E. O., and Islam, S. (2018) ‘Suppressive Effects of Clerodendrum volubile P Beauv. [Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications’, *Frontiers in Pharmacology*, 9(February): 1–13.

Evans, W. C. (2009) *Trease and Evans Pharmacognosy*. Sixteenth. Edinburgh: Elsevier Saunders.

Farag, M., Ahmed, W. J., Foud, I., and Mohammed, M. S. (2014) ‘The Role of Medicinal Plants in The Treatment of Type-2 Diabetes’, *Advanced Journal of Pharmaceuticie and Life Science Research*, 2(4): 1–8.

Farina, F., Piassi, F. G., Moyses, M. R., Bazzolli, D. M. S, and Bissoli, N. (2010) ‘Glycemic and urinary volume responses in diabetic mellitus rats treated with *Solanum lycocarpum*’, *Applied Physiology, Nutrition, and Metabolism*, 35(1): 40–44.

Fetita, L. S., Sobngwi, E., Serradas, P., Calvo, F., and Gautier, J. F. (2006) ‘Review: Consequences of fetal exposure to maternal diabetes in offspring’, *Journal of Clinical Endocrinology and Metabolism*, 91(10): 3718–3724.

Fröde, T. S. and Medeiros, Y. S. (2008) ‘Animal models to test drugs with potential antidiabetic activity’, *Journal of Ethnopharmacology*, 115(2): 173–183.

Fu, L. M. and Li, J. T. (2011) ‘A systematic review of single Chinese herbs for Alzheimer’s disease treatment’, *Evidence-based Complementary and Alternative Medicine*, 2011.

Gad, S. C., Cassidy, C. D., Aubert, N., Spainhour, B., and Robbe, H. (2006) 'Nonclinical vehicle use in studies by multiple routes in multiple species', *International Journal of Toxicology*, 25(6): 499–521.

Gaikwad, S. B., Krishna, M. G. and Rani, S. M. (2014) 'Phytochemicals for Diabetes Management', *Pharmaceutical Crops*, 5(1): 11–28.

Gandhi, G. R., Ignacimuthu, S. and Paulraj, M. G. (2011) 'Solanum torvum Swartz. fruit containing phenolic compounds shows antidiabetic and antioxidant effects in streptozotocin induced diabetic rats', *Food and Chemical Toxicology*. Elsevier Ltd, 49(11): 2725–2733.

Ghorbani, A. (2014) 'Clinical and experimental studies on polyherbal formulations for diabetes: Current status and future prospective', *Journal of Integrative Medicine*. 12(4): 336–345.

Gnana Sundari, S., Rekha, S. and Parvathi, A. (2013) 'Phytochemical evaluation of three species of solanum L.', *International Journal of Research in Ayurveda and Pharmacy*, 4(3): 420–425.

Grover, J. K., Yadav, S. and Vats, V. (2002) 'Medicinal plants of India with anti-diabetic potential', *Journal of Ethnopharmacology*, 2(4): 81–100.

Gupta, A. K., Ganguly, P., Majumder, U. K., and Ghosal, S. (2009) 'Antidiabetic and Antihyperlipidaemic Effects of *Solanum Xanthocarpum* Total Extract In Alloxan Induced Diabetic Rats', *Pharmacology online*, 1: 484–497.

Harborne, J. B. (1973) *Phytochemical methods - A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall.

Hsu, Y. J., Lee, T., Chang, C. L., Huang, Y., and Yang, W. (2009) 'Anti-hyperglycemic effects and mechanism of Bidens pilosa water extract', *Journal of Ethnopharmacology*, 122(2): 379–383.

Idakwoji, P. A., Salawu, O. A., Maiha, B. B., Obidike, I., and Tijani, A. Y. (2015) 'Co-

administeration of Ethanolic Leaf Extract of Moringa oleifera and Metformin Improves Glucose, Lipid and Protein Profiles of Diabetic Wistar rats', *Biokemistri*, 27(3): 123–138.

IDF (2013) *Diabetes Atlas, 2013*. Sixth. Brussels: International Diabetes Federation.

IDF (2017) *Diabetes Atlas, 2017*. Eighth. Brussels: International Diabetes Federation.

Ighodaro, O. M., Adeosun, A. M. and Akinloye, O. A. (2017) 'Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies', *Medicina (Lithuania)*. The Lithuanian University of Health Sciences, 53(6): 365–374.

Inzucchi, S. E. (2002) 'Oral Antihyperglycemic Therapy for Type 2 Diabetes Scientific Review', *Scientific Review and Clinical Applications*, 287(3): 360–372.

Kamatenesi-mugisha, M. and Oryem-origa, H. (2007) 'Medicinal plants used to induce labour during childbirth in western Uganda', *Journal of Ethnopharmacology*, 109(May 2006): 1–9.

Kandimalla, R., Kalita, S., Choudhury, B., and Kotoky, J. (2016) 'a Review on Anti-Diabetic Potential of Genus Solanum (Solanaceae)', *Journal of Drug Delivery and Therapeutics*, 5(1): 24–27.

Kar, D. M., Maharana, L., Pattnaik, S., and Dash, G.K. (2006) 'Studies on hypoglycaemic activity of Solanum xanthocarpum Schrad. & Wendl. fruit extract in rats', *Journal of Ethnopharmacology*, 108(2): 251–256.

Karthikeyan, M. and Balasubramanian, T. (2016) 'In-vivo Animal Models and In-vitro Techniques for Screening Antidiabetic Activity', *Journal of Developing Drugs*, 05(02): 1–6.

Kasali, F. M., Masunda, A. T., Madarhi, J. K., Matambura, A. B., Tshibangu, D. S., Ngbolua K., Kadima, J. N., and Mpiana, P. T. (2016) 'Assessment of antidiabetic activity and acute toxicity of leaf extracts from Solanum nigrum L. (Solanaceae) in guinea-pigs', *International Journal of Herbal Medicine*, 4(6): 14–19.

Kong, J. M., Goh, N., Chia, L., and Chia, T. (2003) 'Recent advances in traditional plant drugs and orchids.', *Acta pharmacologica Sinica*, 24(1): 7–21.

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., Asadi-Samani, M. (2016) 'The role of medicinal plants in the treatment of diseases: A systematic review of Calendula officinalis', *Electronic Physician*, 8(14): 92–95.

Koyama, Y. and Brenner, D. A. (2017) 'Liver inflammation and fibrosis', *The Journal of Clinical Investigation*, 127(1): 55–64.

Kumar, P. J. and Clark, M. L. (2012) *Kumar and Clark's Clinical Medicine*. Eighth Edi, *Elsevier Saunders*. Eighth Edi. Elsevier Saunders.

Kumar, S., Singh, R., Vasudeva, N., and Sharma, S. (2012) 'Acute and chronic animal models for the evaluation of anti-diabetic agents', *Cardiovascular Diabetology*, 11(9): 1–13.

Kwon, Y. I., Apostolidis, E. and Shetty, K. (2008) 'In vitro studies of eggplant (Solanum melongena) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension', *Bioresource Technology*, 99(8): 2981–2988.

Lawal, I. O., Uzokwe, N. E., Igboanugo, A. B. I., Adio, A. F., Awosan, E. A., Nwogwugwu, J. O., Faloye, B., Olatunji, B. P. and Adesoga, A. A. (2010) 'Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria', *African Journal of Pharmacy and Pharmacology*, 4(1): 1–7.

Lenzen, S. (2008) 'The mechanisms of alloxan- and streptozotocin-induced diabetes', *Diabetologia*, 51(2): 216–226.

Liro, A. (1985) 'Variation in weights of body and internal organs of the field mouse in a gradient of urban habitats', *Acta Theriologica*, 30: 359–377.

Lunyera, J., Wang, D., Maro, V., Karia, F., Boyd, D., Omolo, J., Patel, U. D., and Stanifer, J. W. (2016) 'Traditional medicine practices among community members with diabetes mellitus in Northern Tanzania : an ethnomedical survey', *BMC Complementary and Alternative*

Medicine. 16(282).

Ma, L., Zhang, S. and Du, M. (2015) 'Cordycepin from Cordyceps militaris prevents hyperglycemia in alloxan-induced diabetic mice', *Nutrition Research*, 35(5): 431–439.

Manandhar Shrestha, J. T., Shrestha, H., Prajapati, M., Karkee, A., and Maharjan, A. (2017) 'Adverse Effects of Oral Hypoglycemic Agents and Adherence to them among Patients with Type 2 Diabetes Mellitus in Nepal', *Journal of Lumbini Medical College*, 5(1): 34–40.

Markom, M., Hasan, M., Daud, W., Singh, H., and Jahim, J. (2007) 'Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: Effects of solvents and extraction methods', *Separation and Purification Technology*, 52(3): 487–496.

Moshi, M. J., Uiso, F.C., Mahunnah, R.L.A., Malele, S.R. and Swai, A.B.M. (1997) 'A Study of the Effect of Phyllanthus Amarus Extracts on Blood Glucose in Rabbits', *International Journal of Pharmacognosy*, 35(3): 167–173.

Moshi, M. J., Uiso, F.C., Mahunnah, R. L A., Mbwambo, Z. H. and Kapingu, M. C. (2000) 'A Survey Of Plants Used By Tradtional Healers In The Management Of Non-Insulin Dependent Diabetes Mellitus', *East and Central African Journal of Pharmaceutical Sciences*, 3:30–39.

Moshi, M. J., Mbwambo, Z. H., Nondo, R. S. O., Masimba, P. J., Kamuhabwa, A., Kapingu, M. C., Thomas, P., and Richard, M. (2006) 'Evaluation of ethnomedical claims and brine shrimp toxicity of some plants used in Tanzania as traditional medicines', *African Journal of Traditional, Complementary and Alternative Medicines*, 3(3): 48–58.

Moshi, M. J. and Mbwambo, Z. H. (2002) 'Experience of Tanzanian Traditional Healers in the Management of Non-insulin Dependent Diabetes Mellitus', *Pharmaceutical Biology*, 40(7): 552–560.

Moshi, M. J., Otieno, D. F. and Weisheit, A. (2012) 'Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba

District', *Journal of Ethnobiology and Ethnomedicine*, 8(1): 14.

Muntean, E., Michalski, R., Muntean, N., and Duda, M. (2016) 'Chemical Risk Due To Heavy Metal Contamination Of Medicinal Plants', *Hop and Medicinal Plants*, 24(1): 71–78.

Mwakigonja, A., Pak, F., Pyakurel, P., Kaaya, E., Urassa, W., Mosha, I., and Biberfeld, P. (2007) 'Oral Kaposi's sarcoma in Tanzania: Presentation, immunopathology and human herpesvirus-8 association', *Oncology Reports*, 17: 1291–1299.

Navarro, D., Ferreira, A. C., Viana, H., Carvalho, F., and Nolasco, F. (2017) 'Cavernous sinus thrombosis in a patient with nephrotic syndrome', *CEN Case Reports*. Springer Japan, 6(2): 136–139.

Ng'uni, T., Klaasen, J. A. and Fielding, B. C. (2018) 'Acute toxicity studies of the South African medicinal plant Galenia africana', *Toxicology Reports*. Elsevier, 5(July 2017): 813–818..

Niamat, R., Khan, M., Khan, K., Ahmed, M., Mazari, P., Ali, B., Mustafa, M., and Zafar, M. (2012) 'A Review on Zizyphus as Antidiabetic', *Journal of Applied Pharmaceutical Science*, 02(03): 177–179.

Njoku, O. V. and Obi, C. (2009) 'Phytochemical constituents of some selected medicinal plants', *African Journal of Pure and Applied Chemistry*, 3(11): 228–233.

Okokon, J. E., Nwafor, P. A., Okokon, P. J., Umoh, E. E., and Udobang, J. A. (2011) 'Antidiabetic and Hypolipidemic Activities of Ethanolic Root Extract of Croton zambesicus on Alloxan Induced Diabetic Rats', *Asian Journal of Pharmaceutical and Biological Research*, 1(4): 493–499.

Organization for Economic Co-operation and Development (2001) 'Acute Oral Toxicity – Acute Toxic Class Method 423', *OECD Guideline for Testing of Chemicals*, (December): 1–14.

Osadebe, P., Odoh, E. and Uzor, P. (2014) 'Natural Products as Potential Sources of

Antidiabetic Drugs', *British Journal of Pharmaceutical Research*, 4(17): 2075–2095.

Ozougwu, J. C., Obimba, K. C., Belonwu, C. D., and Unakalamba, C. B. (2013) 'The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus - Review', *Journal of Physiology and Pathophysiology*, 4(4): 46–57.

Parasuraman, S., Hoong, S., Zou, L., Wei, D. L., and Loshini, S. (2018) 'Lack of Antidiabetic Effect of Ethanolic Extract of Leaves of *Solanum trilobatum* Linn (Solanaceae)', *Journal of Pharmaceutical Negative Results*, (9): 49–54.

Pariyani, R., Safinar, I., Azam, A., Abas, F., Shaari, K., and Sulaiman, M. (2015) 'Phytochemical Screening and Acute Oral Toxicity Study of Java Tea Leaf Extracts', *BioMed Research International*, 2015: 1–8.

Pin, K., Abdullah, L.C., Ahmad, A.R., Pisar, M., Jamaludin, F., Subramaniam, V. and Mat, A. R. (2010) 'Antioxidant And Anti-inflammatory Activities of extracts of betel leaves (Piper betle) from solvents with different polarities', *Journal of Tropical Forest Science*, 22(4): 448–455.

Poongothai, K., Ponmurugan, P., Ahmed, K., Kumar, B. S., and Sheriff, S. A. (2011) 'Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats', *Asian Pacific Journal of Tropical Medicine*. Hainan Medical College, 4(10): 778–785.

Radenković, M., Stojanović, M. and Prostran, M. (2016) 'Experimental diabetes induced by alloxan and streptozotocin: The current state of the art', *Journal of Pharmacological and Toxicological Methods*. Elsevier B.V., 78: 13–31.

Raj, R., Sahay, S. and Tripathi, J. (2016) 'Medications of diabetes mellitus and antidiabetic medicinal plants: A review', *International Journal of Indigenous Herbs and Drugs*, 1(1): 19–28.

Rani, S. and Kumar, B. (2015) 'Glycemic control efficacy of Phyllanthus niruri . Linn extract

in Diabetic Mice model', *International Journal of Current Microbiology and Sciences*, 4(4): 599–609.

Renzaho, A. M. (2015) 'The post-2015 development agenda for diabetes in sub-Saharan Africa: challenges and future directions', *Glob Health Action*, 8: 27600.

Reusch, J. E. B. and Manson, J. A. E. (2017) 'Management of type 2 diabetes in 2017 getting to goal', *JAMA - Journal of the American Medical Association*, 317(10): 1015–1016.

Rios, J. L., Francini, F. and Schinella, G. R. (2015) 'Natural Products for the Treatment of Type 2 Diabetes Mellitus', *Planta Medica*, 2015(81): 975–994.

Saad, B., Zaid, H., Shanak, S., and Kadan, S. (2017) *Anti-diabetes and anti-obesity medicinal plants and phytochemicals: Safety, efficacy, and action mechanisms*. Cham, Switzerland: Springer Nature/Springer International Publishing.

Saleem, U., Amin, S., Ahmad, B., Azeem, H., Anwar, F., and Mary, S. (2017) 'Acute oral toxicity evaluation of aqueous ethanolic extract of Saccharum munja Roxb. roots in albino mice as per OECD 425 TG', *Toxicology Reports*. Elsevier, 4(October): 580–585.

Shah, S. S., Manigauha, A. and Dubey, B. (2019) 'Formulation and Evaluation of Antidiabetic and Antihyperlipidemic Activities of Polyherbal Formulation in Streptozotocin induced diabetic rat', Pharmaceutical and Biosciences Journal, 7(1): 26.

Singh, M. P. and Pathak, K. (2015) 'Animal models for biological screening of anti-diabetic drugs: An overview', *European Journal of Experimental Biology*, 5(5): 37–48.

Slijepcević, G. R. M. (1991) 'The tannins of the root bark of Sarcopoterium Hypoglycemic Activity of Triterpenes and Tannins from Sarcopoterium spinosum and two Sanguisorba Species Isolation and Testing of the Calcium Blocking Activity of Furanocoumarins from Angelica archangelica', (2):57–58.

Son, L. H. and Yen, P. T. H. (2014) 'Preliminary phytochemical screening, acute oral toxicity and anticonvulsant activity of the berries of Solanum nigrum Linn', *Tropical Journal of*

Pharmaceutical Research, 13(6): 907–912.

Suttie, A. B. (2006) "Pathology of the spleen". *Toxicologic Pathology*. (09): 73195-1.

Tafesse, T. B., Hymete, A., Mekonnen, Y., and Tadesse, M. (2017) 'Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice', *BMC Complementary and Alternative Medicine*, 17(1): 1–9.

Tanko, Y., Mabrouk, M. A., Adelaiye, A. B., Fatihu, M. Y., and Musa, K. Y. (2011) 'Anti-diabetic and some haematological effects of ethylacetate and n-butanol fractions of Ganoderma lucidum aqueous extract in alloxan - induced diabetic wistar rats', *Journal of Diabetes and Endocrinology*, 2(1): 1–7.

Trojan-Rodrigues, M., Alves, T. L.S., Soares, G. L.G., and Ritter, M. R. (2012) 'Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil', *Journal of Ethnopharmacology*. Elsevier Ireland Ltd, 139(1): 155–163.

Umamageswari, M. S., Karthikeyan, T. M. and Maniyar, Y. A. (2017) 'Antidiabetic activity of aqueous extract of Solanum nigrum linn berries in alloxan induced diabetic wistar albino rats', *Journal of Clinical and Diagnostic Research*, 11(7): FC16–FC19.

Vaghasiya, Y. K., Shukla, V. J. and Chanda, S. V. (2011) 'Acute Oral Toxicity Study of Pluchea arguta Boiss Extract in Mice', *Journal of Pharmacology and Toxicology*, 6(2): 113–123.

van de Venter, M., Roux, S., Bungu, L. C., Louw, J., Crouch, N. R., Grace, O. M., Maharaj, V., Pillay, P., Sewnarian, P., Bhagwandin, N., and Folb, P. (2008) 'Antidiabetic screening and scoring of 11 plants traditionally used in South Africa', *Journal of Ethnopharmacology*, 119(1): 81–86.

Walum, E. (1998) 'Acute oral toxicity', *Environmental Health Perspectives*, 106(2): 497–503.

Weese, T. L. and Bohs, L. (2007) 'A Three-Gene Phylogeny of the Genus Solanum (Solanaceae)', *Systematic Botany*, 32(2): 445–463.

West, C., Ploth, D., Fonner, V., Mbwambo, J., Fredrick, F., and Sweat, M. (2016) 'Developing a Screening Algorithm for Type II Diabetes Mellitus in the Resource-Limited Setting of Rural Tanzania', *American Journal of the Medical Sciences*. Elsevier, 351(4): 408–415.

WHO Expert Committee (1999) 'Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications', 1–59.

Yousaf, Z., Wang, Y. and Baydoun, E. (2013) 'Phytochemistry and pharmacological studies on Solanum torvum Swartz', *Journal of Applied Pharmaceutical Science*, 3(4): 152–160.

https://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=150740

APPENDICES

INVESTIGATION TOOLS

Table 9: Observation Chart for Antidiabetic Studies – OGTT Model

DATE: …… **ANIMAL: Albino Mice**

TEST DRUG: …….. **DOSE: ……** **CONC: ……**

SOLVENT: …….. **ROUTE OF ADMINISTRATION: ……..**

PARTICULARS	ANIMALS				
	1	2	3	4	5
Weight of animals (g)					
The volume of drug to be given (ml)					
Time of giving extract/drug/solvent					
Time of giving oral glucose 1g/kg bwt (30min after extract/drug/solvent)					
Blood glucose in mmol/L at;					
FBG					
30 min after oral glucose load					
1 hour after oral glucose load					
2 hours after oral glucose load					
3 hours after oral glucose load					

Table 10: Observation Chart for Antidiabetic Studies – Alloxan Model

DATE: …… **ANIMAL: ….** **TEST DRUG: ……..**

SOLVENT: …….. **DOSE: ……..** **CONC: ……**

ROUTE OF ADMINISTRATION: ……

PARTICULARS		**ANIMALS**									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Weight of animals (g)											
Volume of drug to be given (ml)											
FBG in mmol/L before Alloxan admin (then administer Alloxan)											
Blood glucose in mmol/L at;											
Day 1	FBG (mmol/l)										
	Weight (g)										
Day 5	FBG (mmol/l)										
	Weight (g)										
Day 10	FBG (mmol/l)										
	Weight (g)										
Day 15	FBG (mmol/l)										
	Weight (g)										
Day 20	FBG (mmol/l)										
	Weight (g)										

Table 11: Observation Chart for Acute Oral Toxicity – Toxicity Signs

DATE: …… **ANIMAL: Albino mice** **SOLVENT: ……**

TEST DRUG: …….. **DOSE: ……** **CONC: ……**

ROUTE OF ADMINISTRATION: …… **Time of Onset of Signs …** **Time of Recovery ……**

Toxicity signs;	DAYS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Behavioral Changes;														
(a) Tremors														
(b) Convulsions														
(c) Salivation														
(d) Diarrhoea														
(e) Lethargy														
(f) Sleep														
Respiratory Changes														
Mortality														

Table 12: Observation Chart for Acute Oral Toxicity – Body Weight

ACUTE TOXICITY TEST - BODY WEIGHT RECORDING SHEET

Date …… Animal …… Test Drug ……

Route Of Administration …… Solvent …… Conc ……

GROUPS	TREATMENTS	WEIGHT OF MICE (g)					
		MICE No.	Day 1	Day 3	Day 7	Day 10	Day 14 (Sacrifice Day)
Control	5% Ethanol	1					
		2					
		3					
		4					
		5					
Testing Dose	2000mg/kg	1					
		2					
		3					
		4					
		5					