EFFECT OF EXTRACT-FRACTIONS OF *BALANITES AEGYPTIACA* LEAVES, STEM-BARK AND FRUIT-MESOCARP ON GLUCOSE METABOLIC ENZYMES IN DIABETIC RATS

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULLFILLMENT FOR THE AWARD OF THE DOCTOR OF PHILOSOPHY DEGREE IN BIOCHEMISTRY

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ZARIA, NIGERIA

NOVEMBER, 2018

DECLARATION

I hereby declare that this thesis entitled '**Effect of Extract Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Glucose Metabolic Enzymes in Diabetic Rats**' has been performed by me in the Department of Biochemistry under the supervision of Prof. K.M Anigo, Prof. I.A. Umar and Dr (Mrs) J.O Alegbejo. The information derived from the literature has been duly acknowledged in the text and a list of references provided. To the best of my knowledge and belief, no part of this thesis report was previously presented for another degree or diploma at any University.

MhyaDaniel HASSAN ________________________ _____________________
Signature Date

CERTIFICATION

This thesis titled: **EFFECT OF EXTRACT FRACTIONS OF*BALANITES AEGYPTIACA* LEAVES, STEM-BARK AND FRUIT-MESOCARP ON GLUCOSE METABOLIC ENZYMES IN DIABETIC RATS**by MhyaDaniel HASSAN meets the regulation governing the award of Doctor of philosophy Degree in Biochemistry of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Thesis is dedicated to Almighty God,my beloved wife Mrs. Grace Daniel and children; Matthew and Agnes.

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ABSTRACT

Balanites aegyptiaca fruit and seeds has been reported as useful source of hypoglycemic remedy for the management of diabetes. The leaves and stem-bark are used by traditional medicine practitioners in the management of diabetes mellitus and some ailments. But, very few or no scientific study has been conducted to ascertain their antidiabetic potentials. In this study, antihyperglycemic potential of extracts from *Balanites aegyptiaca*parts were assessed on the activities of glucose metabolic enzymes in streptozotocin induced diabetic rats as a step toward identifying hidden potent compounds and possible mode of actions. Ethanol crude extracts of *Balanites aegyptiaca*leaves, fruit-mesocarp and stem-bark were fractionated with water and ethyl acetate (1:1 v/v) then separated to obtain the specific extract-fractions. Ethyl acetate and aqueous fractions obtained were subjected to acute toxicity study, and then ascertained for antidiabetic effect in STZ-induced diabetic rats. The most potent extract-fraction of *Balanites aegyptiaca* part was further fractionated and subfractions obtained were evaluated in STZ-induced diabetic rats followed by determining possible active ingredients in the subfraction by FTIR and GC-MS. Results of the acute toxicity study showed that fractionated ethanol extracts of*Balanites aegyptiaca* parts are relatively safe at a concentration up to 4000 mg/kg body wt. Rats induced diabetes were characterized by low serum insulin, hyperglycemia and decrease in body weights.Treatment with the ethanol extract-fractions of *Balanites aegyptiaca* parts lowered fasting blood glucose levels, reversed serum total cholesterol, triglyceride, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, high density lipoprotein levels, serum albumin, total protein and

regulate glucose metabolic enzymes activities. The fruit-mesocarp and leaves aqueous fractions were more comparable in lowering fasting blood glucose by 18.61 % and 15.87 % when compared to 24.62 % by metformin. In comparable of the glucose enzymes regulatory effect, the leaves aqueous fraction was more comparable to metformin.The fruit-mesocarp aqueous fraction significantly enhanced glucose 6-phosphodehydrogenase (from 1.40±0.02 to 2.10±0.01 U/min/mg protein) and glucokinase (from 2.22±0.02 to 3.58±0.05 U/min/mg protein). Whereas, leaves aqueous fraction and its methanol subfractionsignificantly suppressed fructose 1,6-bisphosphatase (from 2.19±0.25 to ≤ 1.53±0.11 U/min/μmole Pi liberated) and glycogen phosphorylase (from 3.82±0.21to ≤ 2.76±0.02 U/min/mg protein) but enhanced phosphofructokinase (from 2.06±0.07 to ≥ 2.52±0.03 U/min/mg protein) and glycogen synthase (from 9.41±0.34 x 10^{-2} to ≥ 14.45±0.16 x 10^{-2} U/min/mg protein).Possible active ingredients of methanol subfraction are springol (0.12 %), isoeugenol (0.12 %), eugenol (0.23 %), and 4-vinylguaiacol (0.67 %).In conclusion,fractionated ethanol extracts of *Balanites aegyptiaca* parts are relatively safe, exerted antihyperglycemic, antilipidemicand controlled diabetic-induced alterations of glucose metabolic enzymesin STZ-induced diabetic rats. Theleavesextract of *Balanites aegyptiaca*exert antidiabetic activity as comparable to the fruit-mesocarp. Further research is therefore needed to isolate the compounds in the plant leaves and compare their antihyperglycemic effect with available synthetic ones.

TABLE OF CONTENT

CONTENT PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVATIONS

Abs.	Absorbance
ADP	adenosine diphosphate
AFF	Aqueous fruit-mesocarp fraction
ALF	Aqueous leaves fraction
ALT	Alanine transaminase
AMPK	Adenosine monophosphate activated protein kinase
ASF	Aqueous stem-bark fraction
AST	Aspartate transaminase
cAMP	cyclic adenosine monophosphate
Cm	Centimeter
EFF	Ethyl acetate fruit-mesocarp fraction
ELF	Ethyl acetate leaves fraction
ESF	Ethyl acetate stem-bark fraction
FBG	Fasting blood glucose
F16Bpase	Fructose-1,6-bisphosphatase
FTIR	Fourier Transform Infra-Red
G6P	Glucose-6-phosphate
G6pase	Glucose-6-phosphatase
G6PDH	Glucose-6-phospatedehydrogenase
GC-MS	Gas Chromatography Mass Spectroscopy
GK	Glucokinase
GLp-1	Glucagon-like polypeptide

GluT2	Glucose transporter 2
GluT4	Glucose transporter 4
HMG-Coa	3-Hyroxyl-3-methyl-glutarate Co-a Reductase
L1	Diethyl ether leaves subfraction
L2	Diethyl ether/chloroform leaves subfraction
L3	Chloroform leaves subfraction
L4	Methanol leaves subfraction
LDH	Lactate dehydrogenase
Min.	Minutes
PEP	Phosphoenol pyruvate
PEPCK	Phosphoenolpyruvate carboxyl kinase
PGLUM	Phosphoglucomutase
Pi	Inorganic phosphate
PK	Pyruvate kinase
RF	Retention factor
STZ	Streptozotocin
TCA	Triacetic acid
UV	Ultraviolet
Wt.	Weight
β-NAD^+	Beta-nicotinamide adenine dinucleotide (oxidized)
β-NADH	Beta-nicotinamide adenine dinucleotide (reduced)
β-$NADP^+$	Beta-nicotinamide adenine dinucleotide phosphate (oxidized)
β-NADPH	Beta-nicotinamide adenine dinucleotide phosphate (reduced)

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Diabetes mellitus(DM) is a group of metabolic disorders. It is one of the key health problem, affecting millionsof people worldwide with high frequency rate and is projected to reach 592 million people in the year 2035 (Guariguata *et al*., 2014). There are several types of diabetes mellitus, but the two most common types are Type 1 and Type 2 diabetes mellitus. Type 1 diabetes mellitusis caused by an auto-immune response leading to a breakdown of insulin producing cells (Delmastro and Piganelli, 2011). The pathogenesis of Type 2 diabetes mellitus differs from Type 1 diabetes mellitus, two key features are important; insulin resistance and beta-cell failure which resulted fromaprogressive insulin secretory defect on the background of insulin resistance (NDIC, 2011). Diabetes mellitusis distinguished by a very high level of glucose in the blood(**h**y**pergl**y**cemia)**.

Hyperglycemia in DM largely results from hepatic glucose over production associated with glucose metabolic disorder which occurs as a result of disturbance in the activities of enzymes involved in glycolysis, gluconeogenesis, glycogenesisand glycogenolysis (Soliman *et al*. 2013). **Studies have shown that disturbance of carbohydrate metabolism has significant influences on glucose homeostasis (Abdollahi *et al*., 2004a; Atefi *et al*., 2005).** Deficiency of insulin disrupts**carbohydrate metabolism b**ysuppressing the activities of glycolytic and glycogenic enzymes while promoting gluconeogenic and glycogenoly**t**ic enzymes

(Skim *et al*., 1999; Soliman *et al*. 2013).L**iver** plays a major role in the **glucose homeostasis** by

maintaining abalance between its utilizaion and production.

Maintenance of normalglycemia requires matching of glucose utilization and endogenous production. This could be achieve via coordinated regulations of several metabolic pathways; glycolysis, gluconeogenesis, glycogenolysis, and glycogenesis (Hers, 1999; Nordlie *et al*., 1999).Several regulatory enzymes like glucokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpruvate carboxlkinase, glycogen synthase and glycogen phosphorylase play key roles in thesemetabolic pathways (Nordlie *et al*. 1999). **According to Abdollahi *et al* (2004b),any agent with potential to reverse hepatic carbohydrate metabolism might have influence on** enzymes involved in glu**c**ose and glycogen metabolisms**.** Agius (2007) hasreportedthat inhibition of enzymes involvedin gluconeogenesis and glycogenolysis **c**ontributedto the loweringof fasting plasma glucose.

Management of diabetes mellitus with plants seems promising. Scientific studies have confirmed several plants with wide range of pharmacological activities and health benefits in experimental animals (Ong *et al*., 2011; Gupta *et al*., 2012; Williamson, 2013). Such effort has yielded positive results in the past. For instance, the bioactive metformin is derived from *Galega officinalis*, a plant prescribed in medieval time to relieve the symptoms of diabetes mellitus (Andrade-cetto, 2012).

Plants like *Plumbago zeylanica* and *Citrus unshiu*has shownsignificant impacts on diabetes mellitusby modulating the activities of enzymes involes in glucose metabolism (Zarmouh *et al*., 2010; Parka *et al*., 2013). **Sundaram *et al* (2013) have also reported that** green tea was able to reverse hyperglycemia by regulatinghepaticglucose metabolicenzyme activitiesin diabetic rats.In addition, Farsi *et al* (2014) reported that *Ficus deltoidea Jack*. (Moraceae) retards phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase activity by suppressing the transcription of their genes. Similar finding has been reported on *Newbouldia leavis* extract by Kolawole and Akanji(2014).

Studies on medicinal plants have led to the identification andisolation of a number of bioactive compounds.Such compounds have proven effective in the regulating glucose metabolizing enzymes activity in diabetes mellitus**(Srinivasan *et al*., 2014; Birudu *et al* 2015).** For instance, catechins, epicatechins**, and** ferulic were reported to have significant effect onglucokinase and glycogen synthesis in **diabetic rats (Waltner-Law *et al*., 2002; Jung *et al*., 2007). Also, eugenol isolated from Cloves and fisetinwere reported to have** increased hepatic glucokinase activity and glycogen content as well as decreased the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase **in the liver of diabetic rats (Prasath and Subramanian, 2011;Srinivasan *et al*., 2014).**

The plant *'Balanites aegyptiaca Delile*', also known as 'desert date' in English, a member of *Zygophyllaceae* family, is a common plant specie of the dry land areas of Africa and Asia (Hall and Waljer, 1991; Hall, 1992). In Nigeria, it is found mostly in the Northern region. It is known as '*Aduwa*' in Hausa, '*Utazi'* in Igbo, and '*Teji'*

in Yoruba. *Balanites aegyptiaca* has a long history of traditional uses for wide ranges of disease (Chothani and Vegahasiya, 2011). Emphasis of research has been on utilizing medicinal plants that have long and proven history of curing or treating various ailments (Newman and Cragg, 2007).

Balanites aegyptiaca has been reported as antidiabetic medicinal plantwhich has been studied on both diabetic mice and rats (Mansour and Newairy, 2000; Motaal *et al*., 2012). The fruit and seedof *Balanites aegyptiaca* has beenreported as useful source of hypoglycemic remedy for the management of diabetes mellitus(Samir *et al*., 2003; George*et al*., 2006; Eman-Helal*et al*., 2013). The fruit extract was reported to have stimulated insulin secretion (Samir *et al*., 2003; Ezzat *et al*., 2017), inhibited intestinal α-amylase activity *(*Gad *et al*., 2006), and increased muscle basal glucose uptake (Motaal *et al*., 2012) to lowered blood glucose level. The seed extract was reported to have exerted antihyperglycemic effect by ameliorating beta-cell dysfunction (Eman-Helal *et al*., 2013) while Shafik *et al* (2016) suggested antioxidant activity. In a recent studies, it was reported that the leaves extract stimulated erythrocytes glucose uptake in type II diabetic patients (Mahdy *et al*., 2017) while Gawade and Farooqui (2018) reported that it inhibited alpha amylase activity *in vitro*.

Phytochemical investigations of extracts from different parts of *Balanites aegyptiaca* have identified variety of biologically active compounds like flavonoids, phenolics, terpenes, tanins and saponins (Qusti *et al*., 2014; Al-Malki *et al*., 2015; Gidey, 2016). Literature reports showedthat compounds like coumarins and quercetins were found in the leaves, alkaloids and coumarins in the stem-bark while rutins in the fruit and seed kernel among others (Salwa and El Hadidi, 1988; Sarker *et al*., 2000; Shafik *et al*., 2016). Some postulated antidiabetic bioactive compounds are saponins (Kamel, 1991; George*et al*., 2006), phenolic compounds like vanillic and syringic acids as well as flavonoids (rutin), isorhamnetin, rutinoside, aglycone-quercetin(Motaal *et al*., 2012; Al-Malki *et al*., 2015; Shafik *et al*., 2016).

Thesevariety of chemical compounds in *Balanites aegyptiaca*parts mighthave attributed to the different mechanisms postulated to explain how the plant lower blood glucose in diabetic subjects andwhich could be a potential fordiscovering of new chemical that mightbe use as hypoglycemic agent for the management of diabetes mellitus.

1.2 Statement of the Problem

Diabetes mellitus is one of the disorders for which the treatment is still a challenge globally (El-Kaissi and Sherbeeni, 2011) and stable treatment is yet to be discovered.

Severalantidiabetic medicinal plants could have been a useful source of new hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies. But, little is known about their specific active compounds and mode of actions(Eman Helal, 2000).

Balanites aegyptiaca has been reported as antidiabetic medicinal plant (Mansour and Newairy, 2000; Samir *et al*., 2003; Motaal *et al*., 2012; Eman-Helal*et al*., 2013) and compounds like saponins (George*et al*., 2006), rutin (Motaal *et al*., 2012), vanillic and syringic acids (Al-Malki *et al*., 2015), isorhamnetin 3-rutinoside, 3-robinobioside, quercetin-3-glucoside, aglycones quercetin (Shafik *et al*., 2016)were postulated as the active antidiabetic compounds.However, no single compound has been isolated and fully confirmed as antidiabetic agent.

Several studies have indicated *Balanites aegyptiaca* fruit and seeds are useful source of hypoglycemic remedy for management of diabetes mellitus (Samir *et al*., 2003, Gad *et al*., 2006; Helal*et al*., 2013; Al-Malki *et al*., 2015; Shafik *et al*., 2016). The leaves and stem-bark are used by traditional medicine practitioners in the management of diabetes mellitus and some ailments (Chothani and Vegahasiya, 2011; Abubakar *et al*., 2017). But, very few or no scientific study has been conducted to ascertain their antidiabetic potentials.

1.3 Justification/Significance of the Study

Medicinal plants have a long history of traditional uses in the management of diabetes mellitus and have been found to improve diabeticcontrol with reduced side effects compared to synthetic ones (Newman and Cragg 2007; Chothani and Vegahasiya, 2011), and have be supported by the WHO (1980). Therefore, searching for agent from plants for possible discovering of effective, safer and stable treatment is necessary.

In diabetes mellitus, activities of enzymes involved in glycolysis, gluconeogenesis, glycogenolysisand glycogenesisare altered which disturbsmaintenance of normoglycemia (Hers, 1999; Nordlie *et al*., 1999). Regulating the activities of enzymes involved in these glucose metabolic pathways constitutes an approach to glycemic control and probing antihyperglycemic action of agents including some medicinal plants.

Literature reviewed showed none or very few experimental evidence of antidiabetic potential of *Balanites aegyptiaca* leaves and stem-bark extracts despite their used by some traditional medicine practitioners in the management of diabetes mellitus and some ailments (Chothani and Vegahasiya, 2011; Abubakar *et al*., 2017). Hence,the need to ascertain whether these plant partscould exert antidiabetic activity, and identify bioactive compounds and possible mode of actions.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of this study was to assess antihyperglycemic effects of fractionated ethanol extracts of *Balanites aegyptiaca*leaves, stem-bark and fruit-mesocarp on the activities of key glucose metabolic enzymes in streptozotocin (STZ)-induced diabetic rats as a step towards the identification of bioactive components and mode of action.

1.4. 2 Objectives of the study

The specific objectives of the study were to:

I. Determine acute oral toxicity of extractsof *Balanites aegyptiaca*leaves, stem-bark and fruit-mesocarp.

II. Quantify phenolics and flavonoids content of extracts of*Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp.

III. Ascertain the effect of extracts of*Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp on blood glucose and lipid profile in streptozotocin-induced diabetic rats.

IV. Investigatethe effect of extracts of*Balanites aegyptiaca* partson activity of some key hepatic enzymes of glycolysis, gluconeogenesis, glycogenolysis andglycogenesis in streptozotocin-induced diabetic rats.

V. Validate the antihyperglycemic and antihyperlipidermiceffect of subfractions derived from potent extract of *Balanites aegyptiaca* part in streptozotocin-induced diabetic rats.

VI. Evaluate the enzyme regulatory effect of subfractions derived from potent extract of *Balanites aegyptiaca* part on hepatic key enzymes of glycolysis, gluconeogenesis, glycogenolysis and glycogenesis in streptozotocin-induced diabetic rats.

VII. Identify bioactive constituent(s) from potent extract of *Balanites aegyptiaca's*part.

1.5Null Hypothesis

Fractionated ethanol extracts of *Balanites aegyptiaca*partsdonotreversethe activities of enzymes involved in glucose metabolism to exertantihyperglycemic activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitusis a severe health problem that is increasing rapidly worldwide. It is classified into two major Types, which are: Type 1 diabetes mellitus also called insulin dependent diabetes mellitus, and Type 2 diabetes mellitus, called non-insulin dependent diabetes mellitus. Diabetes mellitus is characterized by a very high level of glucose in the body that causes derangement of metabolism (American Diabetes mellitus Association, 2010).

2.1.1Type 1 diabetes mellitus

Type 1 diabetes mellitus (DM) is a multisystem disease with both biochemical and anatomic/structural consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin, which results from the marked and progressive inability of the pancreas to secrete insulin because of autoimmune destruction of the beta cells (Aathira and Jain, 2014).Type 1 DM can occur at any age. Although it frequently arises in juveniles, it can also develop in adults. Those with type 1 DM usually are not obese and usually present initially with diabetic ketoacidosis (DKA).

The immune-mediated recognition of islet β-cells by auto-reactiveT-cells. This subsequently leads to the liberation of pro-inflammatory cytokines and reactiveoxygen species which destroy the pancreatic β-cells in the islets of Langerhansand loss of insulin secretion (Delmastro and Piganelli, 2011). The loss of β-cell mass consequential tothe activation of pro-apoptotic signaling events is increasingly recognized as a causal andcommitted stage in the development of Type 1 diabetes mellitus (Watson and Loweth, 2009).Moreover, pancreatic β-cells are sensitive to cytotoxic damage caused by reactive oxygenspecies as gene expression and activity of antioxidant enzymes such as glutathione peroxidaseactivity are decreased in these cells (Gupta *et al*., 2011; Pastore *et al*., 2012).

The symptoms of this disease are excessive thirst (polydipsia), hunger (polyphagia) and urination (polyurea). Managing Type 1diabetes mellitususually involves daily insulin treatment to sustain the patient’s life (Standl *et al*., 2006). Type 1 diabetes mellitus accounts for 5-10% of all diagnosed cases of diabetes mellitus, andexhibits hyperglycemia as its hallmark. It is caused by pancreatic β-islet cell failure with resultinginsulin deficiency, mortality and risk factors may be autoimmune, genetic, or environmental(American Diabetes mellitus Association, 2004).

2.1.2 Pathophysiology of type 1 DM

Currently, autoimmunity is considered the major factor in the pathophysiology of type 1 DM. In a genetically susceptible individual, viral infection may stimulate the production of antibodies against a viral protein that trigger an autoimmune response against antigenically similar beta cell molecules(Delmastro and Piganelli, 2011).Type 1 DM is the culmination of lymphocytic infiltration and destruction of insulin-secreting beta cells of the islets of Langerhans in the pancreas. As beta-cell mass declines, insulin secretion decreases until the available insulin no longer is adequate to maintain normal blood glucose levels. After 80-90% of the beta cells are destroyed, hyperglycemia develops and diabetes may be diagnosed (Delmastro and Piganelli, 2011).

Approximately 85% of type 1 DM patients have circulating islet cell antibodies, and the majority also have detectable anti-insulin antibodies before receiving insulin therapy. The most commonly found islet cell antibodies are those directed against glutamic acid decarboxylase (GAD), an enzyme found within pancreatic beta cells (Pilia *et al*., 2011).

The prevalence of type 1 DM is increased in patients with other autoimmune diseases, such as Graves disease, Hashimoto thyroiditis, and Addison disease (Pilia *et al*., 2011). Pilia *et al*(2011) reported a high prevalence of islet cell antibodies (IA2) and anti-GAD antibodies in patients with autoimmune thyroiditis. A study by Philippe *et al*(2011) using computed tomography (CT) scans, glucagon stimulation test results, and fecal elastase-1 measurements reported a reduced pancreatic volume in individuals with DM. This finding, which was equally present in both type 1 and type 2 DM, may also explain the associated exocrine dysfunction that occurs in DM.

Polymorphisms of the class II human leukocyte antigen (HLA) genes that encode DR and DQ were reported as the major genetic determinants of type 1 DM. It was found that approximately 95% of patients with type 1 DM have either HLA-DR3 or HLA-DR4. Heterozygotes for those haplotypes are at significantly greater risk for DM than homozygotes. HLA-DQs are also considered specific markers of type

1 DM susceptibility. In contrast, some haplotypes (e.g. HLA-DR2) confer strong protection against type 1 DM (Noble and Valdes, 2011).

2.1.3 Genetic factors associated with type 1 DM

Although the genetic aspect of type 1 DM is complex, with multiple genes involved,

there is a high sibling relative risk (Davies *et al*., 1994). It was reported that a dizygotic twins have 5-6% concordance rate for type 1 DM whereas monozygotic twins shares the diagnosis more than 50% of the time by the age of 40 years (Steck *et al*., 2005; Redondo *et al*., 2008). It was found that a child of a parent with type 1 DM, the risk varies according to whether the mother or the father has diabetes. Children whose mother has type 1 DM have a 2-3% risk of developing the disease, whereas those whose father has the disease have a 5-6% risk. When both parents are diabetic, the risk rises to almost 30%. In addition, the risk for children of parents with type 1 DM is slightly higher if onset of the disease occurred before age 11 years and slightly lower if the onset occurred after the parent’s 11th birthday. Study also reported that the genetic contribution to type 1 DM is also reflected in the significant variance in the frequency of the disease among different ethnic populations. For instance, it was reported that type 1 DM in European populations is prevalent with people from northern Europe than those from Mediterranean regions(Borchers *et al*., 2010).

The genome-wide association studies have identified several loci that are associated with type 1 DM, but few causal relations have been established. The genomic region most strongly associated with other autoimmune diseases, the major histocompatibility complex (MHC), is the location of several susceptibility loci for type 1 DM in particular, class II HLA DR and DQ haplotypes (Corper *et al*., 2000; Erlich *et al*., 2008).

2.1.4 Environmental factors associated with type 1 DM

Extragenetic factors also may contribute. Potential triggers for immunologically mediated destruction of the beta cells include viruses (eg, enterovirus, mumps, rubella, and coxsackievirus B4), toxic chemicals, and exposure to cow's milk in infancy, and cytotoxins(Paronen *et al*., 2000; Yeung *et al*., 2011).

Combinations of factors may be involved. Lempainen *et al*(2012) reported that enterovirus infection were associated with the appearance of type 1 DM–related autoimmunity among children who were exposed to cow's milk before 3 months of age. These results suggestedthat an interaction between the 2 factors and provide a possible explanation for the contradictory findings obtained in studies that examined these factors in isolation(Cardwell *et al*., 2010). One meta-analysis found a weak but significant linear increase in the risk of childhood type 1 DM with increasing maternal age (Henry *et al*., 2011).A study by Simpson *et al*(2011) found that neither vitamin D intake nor 25-hydroxyvitamin D levels throughout childhood were associated with islet autoimmunity or progression to type 1 DM. Early upper respiratory infection was reported to increase risk factor for type 1 diabetes. In an analysis of data on 148 children considered genetically at risk for diabetes, upper respiratory infections in the first year of life were associated with an increased risk for type 1 diabetes (Melville, 2013 Beyerlein *et al*., 2013).All children in the study who developed islet autoimmunity had at least 2 upper respiratory infections in the first year of life and at least 1 infection within 6 months before islet autoantibody seroconversion.

2.1.5 Type 2 diabetes mellitus

The pathogenesis of Type 2 diabetes mellitus differs from Type 1 diabetes mellitus, two key features are important, namely insulin resistance and beta-cell failure (NDIC, 2011). Type 2 diabetes mellitus occurs mostly in people that are 40 years of age and older and have a family history of diabetes mellitus. Type 2 diabetes mellitus phase is when the pancreas secretes insulin, however, the body is partially or absolutely incapable of using the insulin. Individuals with insulin resistance develop Type 2 diabetes mellitus when they do not continue to produce enough insulin to cope with higher demands. This type of diabetes mellitus is treated through diet changes, exercise and a desirable glycemic control (Nathan *et al*., 2005). Diet changes are needed because it helps manage the diabetes mellitus better and it also helps in experiencing a better feeling physically and mentally. Occasionally, oral medication or insulin is required in Type 2 diabetes mellitus.

Haber *et al* (2003) and Goodarzi *et al* (2010) have reported that high concentrations of glucose associated with oxidative stress results in insulin resistance of metabolic target tissues. To determine whether oxidative stress contributes to insulin resistance induced by hyperglycemia *in vivo*, nondiabetic rats were infused with glucose for 6 hours to maintain a circulating glucose concentration of 15mM with and without coinfusion of the antioxidant N-acetylcysteine (NAC), followed by a 2hours hyperinsulinemic-euglycemic clamp. It was observed from the study that high glucose induced a significant decrease in insulin-stimulated glucose uptake which was prevented by NAC (Haber *et al*., 2003; Goodarzi *et al*., 2010).

Adipose tissue and muscle are the most significant tissues participating in the development of insulin resistance. According to Evans *et al* (2003), oxidative stress modifies the signaling pathway within a cell installing insulin resistance. These pathways include nuclear factor-kappa B, p38 mitogen activated protein kinase, NH(2)-terminal Jun kinases/stress-activated protein kinases, hexosamines, and others. In addition, studies have reported that, activation of these same pathways by elevations in glucose and free fatty acid (FFA) levels led to both insulin resistance and impaired insulin secretion. Therefore, it was proposed that hyperglycemia-induced, and possibly FFA-induced, activation of stress pathways plays a key role in the development of not only the late complications in Type 1 and Type 2 diabetes mellitus, but also the insulin resistance and impaired insulin secretion as seen in Type 2 diabetes mellitus (Evans *et al*., 2003).

Thus, a unifying hypothesis have been proposed by Evans *et al* (2002) whereby hyperglycemia and FFA-induced activation of the nuclear factor-kappa B and NH2-terminal Jun kinases/stress-activated protein kinases stress pathways, along with the activation of the advanced glycosylation end-products/receptor for advanced glycosylation end-products, protein kinase C, and sorbitol stress pathways, play a key role in causing late complications in diabetes mellitus, along with insulin resistance and impaired insulin secretion in Type 2 diabetes mellitusmellitus.

2.2 Hepatic Glucose Homeostasis

Diabetes mellitus is characterized by hyperglycemia resulting from impaired carbohydrate metabolism with an absolute or relative lack of insulin (Eliza *et al*., 2009). Insulin is the key regulator of glucose uptake. The rise in insulin level in the body leads to the suppression of hepatic glycogenolysis and stimulates insulin-dependent glucose uptake into muscle, liver and adipose tissue. The rate of glucose uptake into muscle cells is dependent on the action of insulin on the glucose transporter-4 (GLUT-4) (Rose and Richter, 2005). The GLUT-4 receptor is localized in intracellular vesicles, but when insulin binds to its own membrane receptor, GLUT-4 is transported to the plasma membrane to permit glucose uptake into muscle cells.

Liver plays a vital role in maintaining glucose homeostasis and glucose-transporter-2 (GLUT-2); the main facilitative glucose transporter in the liver (Postic *et al*., 2004). GLUT-2 carries out insulin-independent transport of glucose. The equilibrium between extracellular and intracellular glucose concentration is maintained due to the high capacity of GLUT-2 for glucose. Glucose absorption activates hepatic glucokinase (GK) which phosphorylates glucose, trapping it in liver cells for conversion to glycogen during the postprandial state. During fasting, liver produces glucose via glycogenolysis or by gluconeogenesis from non-carbohydrate precursors, such as lactate, amino acids and glycerol (Postic *et al*., 2004).

The rate of glycogenesis is due to phosphorylase which is controlled by hormones whereas the rate of gluconeogenesis is controlled principally by the activities of unidirectional enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (Engstrom, 1978). PEPCK catalyses the conversion of oxaloacetate to phosphoenol pyruvate (PEP), a rate limiting step of gluconeogenesis. Whereas, G6Pase catalyses the final step of gluconeogenesis, the production of free glucose from glucose-6-phosphate (G6P). In addition, the expression of PEPCK and GK the key enzymes of liver gluconeogenesis and glycolysis, is under the control of insulin (Engstrom, 1978). It was reported that insulin also regulates GLUT2 expression in the liver (Granner and Pilkis, 1990). However, glucagon affects glucose metabolism mainly by inducing glycogen breakdown and glucose-6-phosphatase on one hand whereas, on the other hand glucagon contributes to hyperglycemia by inhibiting pyruvate kinase (Postic*et al*.,, 1993).

It was demonstrated that hepatic glucose production is increased at least twofold in patients with diabetes mellitus (Guyton and Hall, 2006). This shows that, diabetic subjects with mild to moderate fasting hyperglycemia (i.e. 140–200 mg/dL), basal hepatic glucose production is increased by approximately 0.5 mg/kg per minute. Consequently, during overnight sleeping hours, the liver of an 80 kg diabetic individual with modest fasting hyperglycemia adds an additional 35 g of glucose to the systemic circulation. The increase in basal hepatic glucose production (HGP) is correlated closely with the severity of fasting hyperglycemia. Thus, in diabetics with overt fasting hyperglycemia (i.e. 140 mg/dL), an excessive rate of hepatic glucose output is the major abnormality that causes elevated fasting plasma glucose levels (DeFronzo and Mandarino, 2009).

2.3 Management of Diabetes Mellitus

The main aim in the management of diabetes mellitus is to maintain blood glucose levels as near to normal as possible, while avoiding hypoglycemia (Kaul *et al*., 2012; Inzucchi *et al*., 2015). The major components of the treatment of DM using pharmacological and non-pharmacological interventions are described below.

2.3.1 Non-pharmacological interventions

Good glycemic control is considered as the cornerstone management in the prevention of diabetic complications (WHO, 2016). Lifestyle modifications are the foundations in the management of DM and include the prescription of healthy diet, regular exercise, the management of stress, and avoidance of tobacco (Tuomilehto, 2009; Khan, 2012). Eating healthily is of fundamental importance as part of diabetes mellitus healthcare behaviour and has beneficial effects on weight, metabolic control and general well-being (Chan and Woo, 2010).

2.3.2 Pharmacological intervention

The main pharmacological interventions for diabetes mellitus management include oral hypoglycemic and insulin therapies. Currently available oral anti-diabetic agents include sulfonylureas, biguanides, α- glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation (Antidiabetic drugs, 2017). Examples of oral hypoglycemic drugs, their mechanism of action and some adverse effects are summarized in Table 2.1.

2.4 Hepatic Targets for Controlling Diabetes Mellitus

The liver contributes to glucose homeostasis through rapid postprandial clearanceof glucose from the portal vein in the absorptive state after a meal.

When blood glucose falls below normal concentrations, glycogen is mobilized, and glucose is produced by glucogenolysis. Whenever blood glucose concentration increases hepatic glucose uptake increases proportionally, stimulating glucokinase and glycogen synthase. Elevated blood glucose concentrations normally increases insulin release and reduces glucagon release, increasing the insulin-to-glucagon ratio, which inactivates glycogen phosphorylase (inhibiting glycogenolysis), activates glycogen synthase (stimulating glycogen synthesis), and increases the concentrations of fructose-1,6-bisphosphatase. These events reduce hepatic

Table 2.1 Oral Hypoglycemic Agents and their Mode of Actions

Drug class	**Drug name**	**Mechanism of action**	**Some adverse effects**
Biguanides	Metformin Phenformin	Reduce gluconeogenesis in the liver (insulin sensitizers)	Diarrhea, abdominal cramps, nausea and increased vomiting
Sulfonylureas	Glimepiride Glipizide Glibenclamide	Increase insulin secretion by pancreatic β- cells (insulin secretagogues) 1	Hypoglycemia,weight gain, increased risk of cardiovascular death
Meglitinides	Repaglinide Nateglinide	Increase insulin secretion by pancreatic β- cells (insulin secretagogues)	Increased risk for infection, headache
Thiozolidinediones	Pioglitazone Rosiglitazone	Increase glucose uptake by skeletal muscles (insulin sensitizers)	Increased cardiovascular risk, oedema, weight gain
Alpha glucosidase inhibitors	Acarbose Migitol	Inhibit carbohydrate absorption in the small intestine	Flatulence, diarrhoea, pneumatosis

Source: Antidiabetic drugs (2017). Retreived from: www. Diabetic.qu.ca/en/understand-about-diabetes mellitus production of glucose and increase hepatic storage of glucose as glycogen.

Modulation of carbohydrate metabolizing enzymes in the liver may serve as alternative approach for controlling diabetes mellitus. Hepatic enzymatic targets for controlling the elevation of blood sugar levels are the inhibitions of glucose-6-phosphatase, fructose-1,6-bisphosphatase, and glycogen phosphorylase (Tahrani *et al*., 2011). For instance,inhibitions of glucose-6-phosphatase have been reported earlier by Agius (2007) and Tahrani *et al* (2011). According to Ugochukwu and Babady (2014), G6PDH gene or enzyme is a glucose modulatory target of several anti-diabetic agents and of some medicinal plants.

2.5Hepatic Action of Oral Antidiabetic Drug:Metformin

Metformin lowers blood glucose levels without causing overt hypoglycemia orstimulating insulin secretion. In a study of the metformin mechanism of action, it was postulated that metformin decreases endogenous glucose production in diabetes mellitus. The increased plasma glucose level was attributed to a threefold increase in the rate of gluconeogenesis, as assessed by nuclear magnetic resonance spectroscopy (Hundal *et al*., 2000).

Metformin decreased hepatic gluconeogenesis by interfering with respiratory oxidation in mitochondria. It suppressed gluconeogenesis from several substrates, including lactate, pyruvate, glycerol, and amino acids. In addition, metformin increases intra-mitochondrial levels of calcium (Ca^{++}), a modulator of mitochondrial respiration (Kirpichnikov *et al*., 2002).

Other hypotheses stated that metformin activates adenosine monophosphate protein kinase (AMPK). AMPK activation causes a variety of downstream biochemical changes, including decreased hepatic fatty acid synthesis. In addition, very low density lipoprotein synthesis decreases as a result of reduced acetyl-CoA carboxylase activity. This results in suppression of hepatic glucose production and increased glucose uptake (Zhou *et al*., 2001).

2.6Medicinal Plants for Diabetes Mellitus Management

Recent scientific investigation has confirmed the efficacy of several anti-diabetic plants some of which are very effective and relatively non-toxic (Piero *et al*., 2011). The hypoglycemic effects of plants or their preparation have been evaluated and confirmed using *in vitro* assays and animal models (Afifi *et al*., 2005; Uchema *et al*., 2009; Ong *et al*., 2011; Gupta *et al*., 2012a; Rebamang *et al*., 2015; Phoboo *et al*., 2015); some have also been evaluated in humans (Herrera-Arellano *et al*., 2004; Jayawardena *et al*., 2005). Most of the plants contain glycosides, alkaloids, terpenoids, phenolics, flavonoids, polysaccharides, and saponins, which are frequently implicated of having anti-diabetic effect (Jung *et al*., 2006).

2.6.1 Mechanism of action of anti-diabetic plants extract

The anti-diabetic activity of medicinal plants depends upon a variety of mechanisms. Medicinal plants with blood glucose lowering effect have the ability to modulate one or more of the pathways that regulate insulin resistance, β-cell function, homeostasis, and glucose absorption (Chang *et al*., 2013). Some of the proposed mechanisms of action of anti-diabetic plants include: stimulation of insulin secretion, inhibition of insulin degradative processes and reduction of insulin resistance (Pulok *et al*, 2006), regenerating or repairing pancreatic β-cells and protecting the destruction of the β- cells (Oh, 2015), stimulation of glycogenesis and hepatic glycolysis (Chawla *et al*., 2013), inhibition of α-amylase/α–glucosidase enzymes (Kazeem *et al*., 2013), inhibition of gluconeogenesis (Agius, 2007) and preventing oxidative stress in pancreatic β-cell dysfunction (Hosseini *et al*., 2015).

2. 6.2 Enzymes modulatory effect of plant extracts

Extractsfrom some medicinal plants acted as inhibitors/stimulators of glucose metabolizingenzymes (Nachar *et al*., 2013). Amongs the medicinal plants are; *Tinospora cordifolia* (Willd.), a plant used in India as a hypoglycemic, has been documented in various systems of medicines due to its use by many patients and its commercial availability. The plant was found to inhibit the enzymes, G6Pase and fructose-1,6-bisphosphatase (Sangeetha *et al*., 2011).

Farsi *et al* (2014) have reported that, '*Ficus deltoidea Jack* (*Moraceae*)' a native wild evergreen shrub in Malaysia and widely distributed in Southeast Asian countries is widely used in traditional medicine to treat diabetes mellitus. Scientific studies revealed that the herb has potential antidiabetic properties (Aminudin *et al*., 2007; Adam *et al*., 2009). It was reported that the plant suppressed the transcription of genes for gluconeogenic enzymes; phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase. In addition, the plant also stimulated the hepatic glucokinase (GK) and peroxisome proliferation activated receptor-y (PPARγ) gene expression, which consequently leads to an increased expression of GLUT-4 (Farsi *et al*., 2014).

Yuan and Piao (2011) have reported that '*Artemisia sacrorum* Ledeb (Asteraceae)' induces phosphorylation of AMPK, the downstream effector of acetyl-CoA carboxylase, and decreases the concentration of cAMP response element-binding protein, a key transcription factor for gluconeogenic enzyme. The plant is used in traditional Asian medicine to treat diverse chronic and acute hepatitis. The study showed thatthe plant substantially inhibited glucose production by downregulating gluconeogenic gene expression of peroxisome proliferation activated receptor-y, coactivator-1a phosphoenolpyruvate carboxykinase, and G6Pase. *Cecropia obtusifolia* Bertol (Cecropiaceae),a plant used in the treatment of diabetes mellitus wasreportedto improve glycemic control by blocking hepatic glucose output (Andrade-Cetto and Vazquez, 2010). It was reported that the plant contains cholorogenic acid, an inhibitor of glucose-6-phosphate translocase. *Melissa officinalis* L (Lamiaceae), a medicinal plant used in perfumes, cosmetics, tea, and food products in many countries. Study has shown that the plant exerted hypogly-cemic effect by stimulating glucokinase activity and inhibiting G6Pase activity (Chung *et al*., 2010).

2.6.3 Plants chemical compounds

Phytochemical compounds like phenolics, flavonoids, stilbenes, lignans and polymeric lignans have been identified in plants (Pandey and Rizvi, 2009; Bahadoran *et al*., 2013). These compounds act as a defense against ultraviolet radiation, oxidants and pathogens (Lattanzio*et al*., 2006; Mirmiran *et al*., 2009).

Phenolic acids include two main classes hydroxybenzoic acid derivatives (protocatechuic acid, gallic acid, p hydroxybenzoic acid) and hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid, coumaric acid, Ferulic acid, sinapic acid); berry fruits, kiwi, cherry, apple, pear, chicory and coffee were reported to have high content of these phenolic acids (Manach *et al*., 2004).

Flavonoids are the most abundant polyphenols in plants and more than 6000 types of these compounds have been identified (Vinayagam and Xu, 2015). There are six subclasses of flavonoids, these are; anthocyanins, flavonols, flavanols, flavanones, flavones and isoflavones. Anthocyanins (cyanidin, pelargonidin, delphinidin, malvidin) are found in the berries family, red wine, red cabbage, cherry, black grape and strawberry. Flavonols, including quercetin, kampferol and myricetin, have been mainly detected in onion, curly kale, leeks, broccoli and blueberries. Isoflavones are other most important flavonoids that include daidzein, genistein and glycitein; soybeans and soy products are the richest sources of these estrogen-like structure compounds as well as apigenin and lueolin in *Aniseed* extract (Pietta *et al*., 2003; Shobha *et al*., 2013).

Lignans, diphenolic components with phytoesterogenic activity, have been found in high concentrations in linseed and other grains and cereals (Adlercreutz, 2007). Stilbenes occur in plant in low quantities; resveratrol, one of the well studied compounds of these groups, is largely detected in grapes and red wine. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury.

Bioavailability of these bioactive components is dependent on preparation processes, gastrointestinal digestion, absorption and metabolism (Scalbert and Williamson, 2000). During absorption, polyphenols is hydrolyzed by the intestinal enzymes or colonic microflora, and then be conjugated in the intestinal cells and later in the liver by methylation, sulfation or glucuronidation (Boocock *et al*., 2007; D'Archivio *et al*., 2010). Polyphenol consequently reach and accumulate in the target tissue and induces biological properties; polyphenol derivates are mainly excreted through bile and urine. Several studies have shown rapid absorption of polyphenol compounds, such as procyanidins, quercetin and flavanols into plasma, with plasma concentrations peaking at 2 or 3 hours after ingestion (Manach *et al*., 2004; D'Archivio *et al*., 2010).

Several biological activities and beneficial properties have been documented for plant chemicals, some of the well known ones include; antioxidant, anti-diabetes mellitus, anti-allergic, anti-inflammatory, anti-viral and anti-microbial, anti-proliferative, anti-mutagenic, anti-carcinogenic, free radical scavenging, regulation of cell cycle arrest, apoptosis, and induction of antioxidant enzymes (Ajayi *et al.*, 2011; Williamson, 2013).

2. 6.4 Antidiabetic activity of plant chemicals

Research on medicinal plants has led to the isolation and identification of a number of bioactive compounds (Perola de Oliveira *et al*., 2011; Abirami and Natarajan, 2014). Phytochemicals from plants origin have been used to treat human and animal diseases due to their effectiveness and lesser side effects (Bahadoran *et al*., 2013).

Hypoglycemic activities of phytochemicals like phenolics mainly attributed to reduce intestinal absorption of dietary carbohydrate, modulation of the enzymes involved in glucose metabolism, improvement of β-cell function and insulin action, stimulation of insulin secretion, antioxidant and anti-inflammatory properties of these components (Prince and Kannan 2006; Bahadoran *et al*., 2013). Studies have reported that some phenolics like; catechins and epicatechins, chlorogenic acids, ferulic acids, caffeic, tannic acids, quercetin and naringenin,interfere with absorption of glucose from intestine via inhibition of Na-dependent glucose transporters (SGLT1 and SGLT2) (Kobayashi *et al*., 2000; Johnston, *et al*., 2005).

Some investigations have shown that phenolics regulate carbohydrate metabolicpathways like glycolysis, glycogenesis and gluconeogenesis usually impaired in diabetes mellitus. Ferulic acid, a hydroxycinnamic acid derivative, effectively suppresses blood glucose by elevating glucokinase activity and production of glycogen in the liver and increased plasma insulin levels in diabetic rats (Jung *et al*., 2007). Treatment of diabetic rats with hesperidin and naringin, two main citrus bioflavonoids, was accompanied with increased hepatic glucokinase activity and glycogen content, attenuated hepatic gluconeogenesis via decrease the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK), and subsequent improvement of glycemic control (Jung *et al*., 2005).

In addition, catechins and epicatechins have been shown to attenuate hyperglycemia and hepatic glucose output via downregulating the expression of liver glucokinase and upregulation of PEPCK genes (Waltner-Law *et al*., 2002). In a similar study, **modulator**y **activities ofeugenol isolated from Cloves, and fisetin (a bioflavonoid found in fruit and vegetable) were reported on carbohydrate metabolizing enzymes in diabetic rats (Prasath and Subramanian, 2011; Srinivasan *et al*., 2014).** Plant chemicals have also been found to influence peripheral glucose uptake in both insulin sensitive and non-insulin sensitive tissues. Study have showed that phenolics stimulated glucose uptake by comparable performance to metformin and thiazolodinedione, main common oral hypoglycemic drugs (Prabhakar and Doble, 2009).

In vitro studies have shown that some polyphenols such as quercetin, resveratrol improved insulin-dependent glucose uptake in muscle cells and adipocytes by translocation of glucose transporter, GLUT4, to plasma membrane mainly through induction of the AMP-activated protein kinase (AMPK) pathway (Park *et al*., 2007; Zhang *et al*., 2011). AMPK, an important sensor of cellular energy status, has a key role in metabolic control; activation of this pathway is considered as a new treatment for obesity, type 2 diabetes mellitus and other metabolic syndromes (Zhou *et al*., 2001; Towler and Hardie, 2007; Ong *et al*., 2011).

Hyperglycemia-induced oxidative stress in pancreatic β-cells plays a pivotal role in the development of diabetes mellitus (Kajimoto and Kaneto, 2004; Drews*et al*., 2010). Oral administration of phenolic-rich chestnut extract in STZ-induced diabetic rats had favorable effects on serum glucose and viability of β–cell through attenuation of oxidative stress, enhancing the natural antioxidant system, and inhibition of lipid peroxidation (Yin *et al*., 2011).

Resveratrol (3, 4, 5-trihydroxystilbene),a phenolic compoundfound in grapes, wine, grape juice, peanuts, and berries; improved glucose tolerance, attenuated β–cell loss, and reduced oxidative stress in pancreatic islet (Szkudelski and Szkudelska, 2011). *Resveratrol* also alleviated chronic over stimulation induced-workload and impose pressure on the β-cells, and subsequently delay the degradation of pancreatic islets and progress of diabetes mellitus. The effect appears to be due to the diminished stimulatory effects of hyperglycemia in insulin secretion; some experimental and *in vitro* studies demonstrated that resveratrol has the potential to reduce insulin secretion through induction of metabolic changes in β-cells (Szkudelski, 2006; Szkudelski and Szkudelska, 2011).

Several mechanisms have been proposed to explain the anti-diabetic action of this stilbene (resveratrol); modulation of SIRT1 which improves whole-body glucose homeostasis and insulin sensitivity (Milne *et al*., 2007; Harikumar and Aggarwal, 2008; Price *et al*., 2012). Treatment with resveratrol delayed the onset of insulin resistance. A possible mechanism was thought to be related to the inhibition of K^+ ATPase and K^+ V channel in beta cells (Chen *et al*., 2007). Resveratrol is able to regulate postprandial glycemia and inhibit the development of glucose intolerance by a facilitated insulin response and attenuated secretion of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like polypeptide-1 (GLP-1) (Dao *et al*., 2011).

A number of studies have demonstrated that phenolics limit the incidence of heart diseases (Nardini *et al*., 2007; Bahadoran *et al*., 2013). One of the most important favorable effects of phenolics on cardiovascular system in diabetes mellitus is improvement of dyslipidemia. Based on research conducted, phenolic compounds such as procyanidins induced hypolipidemic effects by decreasing apolipoprotein B synthesis and secretion, inhibition of cholesterol estrification and intestinal lipo-protein production (Vidal *et al*., 2005).

The hypolipidemic effects of catechins and proanthcyanidins are related to inhibition of key enzymes in lipid biosynthesis pathway and reduced intestinal lipid absorption. Catechins also interact with proteins involved in cholesterol translocation from the enterocyte brush border (ATP-binding cassette proteins, multidrug resistance P-glycoprotein 1, B type1-scavenger receptors, Niemann Pick C-1 like protein), change their function and effectively reduce cholesterol absorption (Koo and Noh, 2007).

Phytochemicals are potent inhibitors of LDL oxidation and this type of oxidation is considered to be a key mechanism in development of atherosclerosis (Aviram *et al*., 2007). Studies have shown that resveratrol and berberine potentially inhibit the oxidation of the LDL particles via chelating copper or by direct scavenging of the free radicals (Cucciolla *et al*., 2007; Shakibaei *et al*., 2009; Wang *et al*., 2011). Phytochemicals protect cardiovascular diseases via anti-platelet, enhancement of the production of vasodilating factors such as nitric oxide, anti-inflammatory effects as well as increasing HDL, and improving endothelial function and inhibiting the synthesis of vasoconstrictor such as endothelin-1 in endothelial cell (Schini-Kerth *et al*., 2010; Lecour and Lamont, 2011).

Phytochemicals exert preventive effects in treatment of asthma. In asthma the airways react by narrowing or obstructing when they become irritated. This makes it difficult for the air to move in and out. This narrowing or obstruction can cause one or a combination of symptoms such as wheezing, coughing, shortness of breath and chest tightness. Epidemiological evidence that phenolics might protect against obstructive lung disease come from studies that have reported negative associations of apple intake with prevalence and incidence of asthma, and a positive association with lung function (Tabak *et al*., 2001; Woods *et al*., 2003).

Intake of phytochemicals is also reported as beneficial in osteoporosis. Supplemen-tation of diet with genistein, daidzein or their glycosides for several weeks prevents the loss of bone mineral density and trabecular volume caused by the ovariectomy (Nakajima *et al*., 2001). Phytochemicals also protect skin damages induced from sunlight. Study on animals provide evidence that polyphenols present in tea, when applied orally or topically, ameliorate adverse skin reactions following UV exposure, including skin damage, erythema and lipid peroxidation (Kim *et al*., 2001). Black tea polyphenols are reported to be helpful in mineral absorption in intestine as well as to possess antiviral activity.

Study has reported that theaflavins present in black tea were found to have anti HIV-1 activity. Theaflavins inhibited the entry of HIV-1 cells into the target cells (Sharma and Rao, 2009). The authors stated that, HIV-1 entry into the target cell involves fusion of glycoprotein (GP) and envelope of the virus with the cell membrane of the host cells. Haptad repeat units present at N and C terminals of GP41 (membrane protein) on the viral envelope, fuse to form the fusion active GP41 core, which is a six-helical bundle. Theaflavins were found to block the formation of this six-helix bundle required for entry of the virus into the host (Sharma and
Rao, 2009).

In addition, Theaflavin 3,3-digallate, and theaflavin 3-gallate were found to inhibit Severe Acute Respiratory Syndrome (SARS) corona virus. This antiviral activity was due to inhibition of the chymotrypsin like protease which is involved in the proteolytic processing during viral multiplication (Sharma and Rao, 2009).

2. 7The Plant *Balanites aegyptiaca*

*Balanites aegyptiaca*known as ‘Desert date or Thorn tree’ in English, is a member of *Zygophyllaceae*, one of the most common plant species of the dry land areas of Africa and South Asia (Hall and Waijer, 1991; Hall, 1992). In Nigeria, it is found mostly in the Northern region. It is known as ‘*Aduwa*’ in Hausa, ‘*Utazi’* in Igbo, and '*Teji'* in Yoruba. It grows to 6 -10 meters in height, is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture. The photographs of *Balanites aegyptiaca* whole plant and the fruits are presented in Plate I and II below.

2.7.1 Traditional medicinal uses of *Balanites aegyptiaca*

Literature review revealed that *Balanites aegyptiaca*has a long history of traditional uses for wide ranges of disease (Chothani and Vegahasiya, 2011). The kernel oil is used for the treatment of wounds in Nigeria (Breye and Brandwijk, 1982). Also, in Nigeria, a mixture of dried leaves powder of *Balanites aegyptiaca* and *Ricinus communis* in water is used for contraception (Oliver-Bever, 1986). In the north-eastern part of Nigeria, the fruit-mesocarp is prepared as pap and taken as remedy against symptoms of diabetes mellitus and other related diseases (personal communication). In Egyptian folk medicine, the fruits are used as an oral hypoglycemic (Kamel, 1991). Aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice (Sarker et al., 2000). The stem-bark, fruits and leaves of *Balanites aegyptiaca* have been reported to be used in the management of diabetes by traditional medical practitioner (Chothani and Vegahasiya, 2011; Abubakar *et al*., 2017).

2.7.2 Pharmacological activities of *Balanites aegyptiaca*

It has been experimentally proved that *Balanites aegyptiaca* possess antioxidant, antimicrobial, anticancer, hypocholesterolemic, antidiabetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, cardioprotective, and antinociceptive properties (Chothani and Vegahasiya, 2011).The bark extract of Balani*tes aegyptiaca* managedAIDS and Leukemia (Hamid *et al*., 2001).

2.7.3 Antidiabetic activity of various parts of *Balanites aegpytiaca*

Balanites aegpytiaca play an important role in the management of diabetes mellitus. Its antihyperglycemic effect has been studied in several diabetic animalsmodel (Mansour and Newairy, 2000; Motaal *et al*., 2012; Nadro and Samson, 2014). The extracts of the fruit-mesocarps and seeds of *Balanites aegpytiaca* has been widely studied and was reported to exhibited potential antihyperglycemic activity and also improved blood cholesterol and triglycerides toward normal levels in diabetic-induced animals (Samir *et al*., 2003; George*et al*., 2006; Eman Helal*et al*., 2013; Al-Malki *et al*., 2015).

Kamel *et al* (1991)had earlier reported that aqueous extract of the fruit mesocarp, as well as the polysaccharide fraction (precipitated by excess of alcohol) and the supernatant (saponin-rich), exerted significant antidiabetic activities in STZ-

Plate I. Photograph of *Balanites aegyptiaca* plant

Plate II. *Balanites aegyptiaca* Fruit

induced diabetic mice. Samir *et al*(2003) reported antidiabetic and antilipidemiceffects of water and ethanolic extracts of the*Balanites aegyptiaca* fruits in normal senile diabetic rats. The*Balanites aegyptiaca* fruit extract was reported to reduceblood glucose level by 24% in STZ-induced diabetic rats (Gad *et al*., 2006). Motaal *et al* (2012) has reported potential antidiabetic activities of different extracts and fractions of the *Balanites aegyptiaca* fruit in cultured C2C12 skeletal muscle cells and 3T3-L1 adipocytes.

The aqueous extract of *Balanites aegpytiaca* seeds has been reported to exert hypoglycemic and hypolipidemic effects, the study showed that it increased insulin level, and decreased insulin resistance (Eman-Helal *et al*., 2013). The antihyperglycemic effect of *Balanites aegyptiaca* seed kernel was reported in alloxan-induced diabetic rats (Nadro and Samson, 2014; Shafik *et al*., 2016).

In a recent *in vitro* studies,the *Balanites aegyptiaca* leaf extracts was reported to possessantidiabetic properties (Madhy *et al*., 2017; Gawade and Farooqui, 2018). Pharmacognostic studies on the effectiveness of *Balanites aegpytiaca* leaves and stem-bark extracts showed thatthey can be use in the treating or prevention of diseases(Gupta *et al*., 2012b; Salve, 2016). In regard to this, literature survey on the use of *Balanites aegyptiaca* leaf and stem-bark for treatment of various ailment was conducted and found that several studies has reported their antimicrobial activities(Doughari *et al*., 2007; Hena *et al*., 2010; Abdulhamid and Sani, 2016). The leaf extract has also be reported to have improved liver parameters in rats (Jacob *et al*., 2017). The leaves and stem-bark were recently reported as the potential parts of *Balanites aegyptiaca* use by traditional medicine practitioners for the management of diabetes mellitus in Zaria, Kaduna State, Nigeria (Abubakar *et al*., 2017). In a recent study *in vitro* antidiabetic activity of the leaf extract was reported (Gawade and Farooqui, 2018).

2.7.4 Some bioactive compounds of *Balanites aegyptiaca*

Phytochemical investigation of *Balanites aegpytiaca* parts has shown that it contained a variety of compounds. It was earlier reported that the leaves contained saponin, furanocoumarine and flavonoids like coumarins and quercetins, alkaloids and coumarins in the stem-bark while rutins was in the fruit among others (Sarker *et al*., 2000; Motaal *et al*., 2012, Gidey, 2016). Studies also reported that the fruit-mesocarp contains protein, sugars, organic acids, other constituents like 3-rutinoside and 3-rhamnogalactoside of isorhamnetin (Salwa andHadidi, 1988), diosgenin (Khare, 2007), and a mixture of 22R and 22S epimers of 26-(Oβ-D-glucopyranosyl)-3-β-[4-O-(β-D-glucopyranosyl)-2-O-(α-L-rhamnopyranosyl)-β-D-luco pyranosy-loxy]-22,26dihydroxy furost-5-ene. The kernel contained xylopyranosyl derivative of the above saponin present in mesocarp (Staek *et al*., 2007) while nine saponins have been reported from the kernel cake (Chapagain and Wiesman, 2007). The leaves and fruit kernels were found to contain six diosgenin glucosides including di-, tri-, and tetraglucosides (Liu and Nakanishi, 1982). The bark contain furanocoumarin bergapten and dihydrofuranocumarin D-marmesin (Seida *et al*., 1981; Sarker *et al*., 2000). It also contains beta-sitosterol, bergapten, marmesin, and beta-sitosterol glucoside (Ansari *et al*., 2006; Breimer *et al*., 2007).

The hypoglycemic properties of *Balanites* aegyptiaca has been attributed to the presence of some of the above mention compounds. Kamel *et al*(1991) found two steroidal saponins from one of the active extract-fraction of the aqueous extract of *Balanites aegyptiaca* fruit after treating a diabetic mice by an oral administration. The study reported that the aqueous extract of the fruit mesocarp, as well as the polysaccharide fraction (precipitated by excess of alcohol) and the supernatant (saponin-rich), exerted significant antidiabetic activities in STZ-induced diabetic mice.

A study conducted by Motaal *et al*(2012) has reported that different extracts and fractions of *Balanites aegyptiaca* fruit in cultured C2C12 skeletal muscle cells and 3T3-L1 adipocytes exerted antidiabetic activities. It was reported that 200 µg/ml of the sugars fraction (A1) showed the highest activity, it increased basal glucose uptake by 52 % in muscle cells; which is twice the activity of 100 nM insulin [insulin equivalent (IE) = 2.0 ± 0.07]. Whereas, dichloromethane (E) and ethyl acetate (F) successive extracts exerted 37 and 41 % increase in the glucose uptake, and accelerated triglyceride accumulation in pre-adipocytes undergoing differentiation, comparably with 10 µM rosiglitazone [rosiglitazone equivalent (RE) was 1.6 ± 0.3 and 0.7 ± 0.1) respectively. Gas chromatography (GC) analysis of the A1 revealed the presence of xylose, rhamnose, sorbitol, fructose, galactose and glucose while extracts E and F when standardized by high-performance liquid chromatography (HPLC) was reported to contained 0.031 and 0.239 % rutin and 0.007 and 0.004 % isorhamnetin, respectively.

Study by Al-Malki *et al* (2015) reported that treating diabetic rats with ethyl acetate extract (EAE) of *Balanites aegyptiaca* fruit at 10, 25 and 50 mg /kg body weight resulted in a significant reduction in blood glucose levels. The GC-MS analysis of EAE of fruits of *Balanites aegyptiaca* revealed the presence of vanillic acid (26.58%) with the molecular formula $C_8H_8O_4$, and MW 312, syringic acid (24.08%) with molecular formula $C_9H_{10}O_5$, and MW 342 and, β-sitosterol (23.94%) with molecular formula $C_{29}H_{50}O$ and MW of 414. Shafik *et al*(2016) reported that, 50 mg/kg body weight of seed kernel of *Balanites aegyptiaca* exerted antihyperglycemic and antilipid peroxidative effects as well as increased activities of enzymatic and non-enzymatic antioxidants in allonxan-induced diabetic rats. Phytochemical investigation of the ethanolic extract of the plant seed kernel using column and preparative paper chromatography resulted in 9 compounds; isorhamnetin 3-rutinoside, 3-robinobioside, 3-0-glucoside, 3-0-galactoside, 3,7-diglucoside, quercetin-3-glucoside, 3-rutinoside, aglycones quercetin, and isorhamnetin respectively.

A study have suggested that isorhamnetin glycosides in plants may be responsible for the antidiabetic effect and also reversed endoplasmic reticulum stress markers and the expression of enzymes regulating lipid metabolism(Rodriguez-Rodriguez *et al*., 2015). Rutin have been shown to stimulate glucose uptake in the rat soleus muscle via the PI3K, a typical protein kinase C and mitogen-activated protein kinase pathways (Kappel *et al*., 2013). Rutin has been reported to significantly improved body weight, reduced plasma glucose and restored the depleted liver antioxidant status and serum lipid profile in HFD/STZ induced diabetic rats (Niture *et al*., 2014).

Recent study has reported some compounds in the leaf extract of *Balanites aegyptiaca*(Gawade and Farooqui, 2018). The compounds identified in Balanites aegyptiaca(L.) leaves following investigation on alpha amylase inhibitory effect were phenol, 2,4-bis(1,1-dimethylethyl)-alpha-D-glucopyranoside, methyl, 1-hexene, 3,5,5-trimethyl, neophytadiene, 1-hexanol, 4-methyl-6-Octen1-ol, 3,7-dimethyl-, propanoate, 16-heptadecenal, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (T-Phytol), 1H-Indene, 1-hexadecyl-2,3-dihydro-, 1-tridecanol, carbonic acid, neopentylcyclo hexyl methyl ester and cyclopentane methanol, alpha-cyclohexyl-2-nitro.

2.7. 5 Antidiabetic mode of actions of *Balanites aegyptiaca*

Studies have shown that *Balanites aegyptiaca* exert antidiabetes, antioxidant, antilipidemia etc. Different variety of biologically active chemicals possess by the plant (Salwa and Hadidi, 1988; Sarker *et al*., 2000; Motaal *et al*., 2012) could have attributed to its different biological functions. In general, It was reported that the hypoglycemic activities of medicinal plants mainly attributed to reduce intestinal absorption of dietary carbohydrate, modulation of the enzymes involved in glucose metabolism, improvement of β-cell function and insulin action, stimulation of insulin secretion, antioxidant and anti-inflammatory(Prince and Kannan, 2006; Bahadoran *et al*., 2013). *Balanites aegyptiaca* seem to act by more than one mechanisms to low blood glucose. The plant extract showed to have oxygen radical scavenging activity, increase insulin secretion, enhanced regeneration of beta cell of the pancreas. It also possess alpha amylase inhibitory activity and enhancescellular glucose uptake, below are details of the above mentioned activities.

Oxygen radical scavenging activity: According to Al-Malki *et al* (2015) and Shafik

et al (2016), *Balanites aegptiaca* extract exert antioxidant activity to combat diabetes mellitus. Al-Malki *et al* (2015) studied the effect of ethyl acetate extract (EAE) of Balanites aegyptiaca fruit (10, 25 or 50 mg/kg body weight) in experimental diabetic rats. Rats were allocated into five groups; control, diabetic, and diabetic rats treated with 10, 25, and 50 µg/kg body weight of EAE for eight weeks. The results reported a protective role of EAE against oxidative stress induced by streptozotocin. It showed that EAE treatment produced a reduction in blood glucose levels, HbA1c, malondialdehyde and vascular endothelial growth factor (VEGF) in diabetic retina as well as enhanced antioxidant capacity against streptozotocin-induced oxidative stress. Al-Malki *et al* (2015) reported that *Balanites aegyptiaca* fruit extract has potential benefits in the prevention of retinopathy in diabetes via inhibition of free radical production and enhancement of antioxidant potential.

Shafik *et al* (2016) reported increased antioxidant status like SOD, catalase, GSHPs and decline in TBARS concentration in diabetic treated rats suggesting its potent antilipid peroxidative and antioxidative effects. About 500 g of Balanites aegyptiaca seeds kernel were dried, powdered and then soaked in 1500 ml of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at 40-50^{0}C under reduced pressure. Diabetic rats were orally administered 50mg/kg body weight of *Balanites aegyptiaca* seeds kernel extract. Results showed oral administration of *Balanites aegyptiaca* seed kernel at a dose of 50 mg/kg body weight significantly exerted antihyperglycemic and antilipid peroxidative effects as well as increased the activities of enzymatic antioxidants and levels of non enzymatic antioxidants

Increase insulin secretion: *Balanites aegyptiaca* extract was reported to have stimulated insulin secretion to lowered blood glucose levels (Samir *et al*., 2003;Ezzat *et al*., 2017). Samir *et al* (2003) has reported that oral administration of both aqueous and ethanolic extract of *Balanites aegyptiaca* significantly increased serum insulin levels in diabetic treated rats. Fruit flesh was used, it was extracted with water and ethanol separately using soxhlet apparatus for about 10 hours. About 80 mg/kg body weight of either the aqueous or ethanolic extracts were administered to the diabetic-induced rats groups for 30 days. Result showed that oral administration of aqueous or ethanolic extracts from Balanites aegyptiaca fruit flesh for 30 days to diabetic rats induced a highly significant decrease ($P< 0.01$) of serum glucose level, increased liver glycogen content, induced significant increase ($P< 0.05$) of serum insulin level but decreased glucagon level. It was suggested that the anti-diabetic effect of *Balanites aegyptiaca* fruit flesh was attributable in part to the increased in serum insulin concentration.

Enzymes regulatory effects (alpha amylase inhibition): In a study on antihyperglycemic effect of *Balanites aegyptiaca* in STZ-induced diabetic rats, it was reported that *Balanites aegyptiaca*could inhibit intestinal α-amylase activity (Gad *et al*., 2006). It was also reported that the activity of some glucose metabolic enzymes: glucose-6-phosphatase and hexokinase could be regulated by extract of *Balanites aegyptiaca*(Gad *et al*., 2006).

In a study by Gawade and Farooqui (2018), *in vitro* inhibition of alpha amylase activity by *Balanites aegyptiaca* leaves extract was reported. The study used *Balanites aegyptiaca*(L.) air dried leaves which was crushed into powdered and about 10 g was mixed in 100 ml of ethanol and kept on a magnetic stirrer for 2 hours and extracted using a soxhlet apparatus sequentially in ethanol. The activity was investigated through the inhibition of α-amylase. Acarbose at a concentration of (20-100μg/ml) was used while ethanol extract at 20-100μg/ml was used. Result showed that acarbose showed α-amylase inhibitory activity from 47.17 to 68.81% with an IC_{50} value 27.90μg/ml, whereas ethanol extract (20-100μg/ml) of *Balanites aegyptiaca* showed potent inhibition activity in a dose dependent manner from 39.96 to 53.02% with an IC_{50} value of 84.08μg/ml.

Enhance cellular glucose uptake: Motaal *et al*(2012) reported that *Balanites aegyptiaca* extract increases muscle basal glucose uptake. Motaal *et al*(2012) stated that the increased muscle basal glucose uptake participated in the traditionally known, and *in vivo* proven, antidiabetic effect of the *Balanites aegpytiaca*. The study suggested that phenolic compounds might have attributed to this activity. In an i*n vitro* studies, it was reported that the plant extract improved insulin-dependent glucose uptake in muscle cells and adipocytes by translocation of glucose transporter, GLUT4, to plasma membrane mainly through induction of the AMP-activated protein kinase (AMPK) pathway (Park *et al*., 2007; Zhang *et al*., 2011). The glucose uptake reported in muscle cells was studied with C2C12 cell line. In another study, *Balanites aegyptiaca* leaves extract was reported to have stimulated erythrocytes glucose uptake in type II diabetic patients (Mahdy *et al*., 2017).

Regeneration of beta cell of the pancreas: A study has reported that *Balanites aegyptiaca* extract was able to ameliorate beta-cell dysfunction (Eman-Helal *et al*., 2013).The study used fifteen adult male albino rats which were divided into two groups; group 1: control group, group 2: alloxan induced diabetic rats that were divided into two subgroups; subgroup1: diabetic untreated rats, subgroup 2: diabetic treated with aqueous extract of *Balanites aegyptiaca* (seeds). Results showed diabetic rats group treated with the plant seeds extract ameliorated most of the toxic effects of alloxan and showed partially improvement in histological changes produced by alloxan. It was concluded that, treating diabetic rats with water extract of *Balanites aegyptiaca* seeds could ameliorate β-cells dysfunction and increase insulin's receptors sensitivity associated with improved in general diabetic conditions. Some of the *Balanites aegyptiaca* compounds with antidiabetic properties and likely mode of actions is summarized in Table 2.2.

Table 2.2 Summary of *Balanites aegyptiacaDel*. Antidiabetic Bioactive Compounds andtheir likelyMode of Actions

Plant Parts	**Type of Extract**	**Bioactive Compounds**	**Mode of Action**	**Reference**
Fruit- mesocarp and epicarp	Aqueous and ethanolic extracts	Steroidal saponins, Isorhamnetin-3-0-robinobioside	--	Kamel, 1991
Fruit flesh	Aqueous and ethanolic extracts	--	Stimulation of insulin secretion	Samir *et al*., 2003
Fruit	Saponin rich-extract	Saponins	--	George *et al*., 2006
Fruit-mesocarp	Aqueous extract	--	Insulinomimetic effect and Inhibition of alpha amylase activity	Gad *et al*., 2006
Fruit-mesocarp	Ethyl acetate and dichloromethane extracts	Rutin and isorhamnetin	Enhanced muscle glucose uptake	Motaal *et al*., 2012
Seed	Aqueous extract	--	Amelioration of β–cell dysfunction	Eman-Helal *et al*., 2013
Seed Kernel	Kernel cake	--	--	Nadro and Samson, 2014
Fruit	--	Trigonelline	--	Farag & Wessjohann, 2015
Fruit-mesocarp	Ethyl acetate extract	Vallinic, syringic and β-sitosterol	Antixoidant activity	Al-Malki *et al*., 2015
Seed Kernel	Ethanolic extract	Isorhamnetin 3-rutino-side, quercetin-3-glucoside	Antioxidant activity	Shafik *et al*., 2016
Fruit-pericarp	Methanolic extract	Furostanol saponin	Stimulationof insulin secretion	Ezzat *et al*., 2017
Leaves	--	--	Stimulation of erythrocyte glucose uptake	Mahdy *et al*., 2017
Leaves	Ethanolic extract	Phenol, 2,4-bis(1,1-dimethyethyl)	Inhibition of alpha amylase activity	Gawade and Farooqui, 2018

-- = not specified

CHAPTER THREE

4.0 **MATERIALS AND METHODS**

4.1 Materials

3.1.1 Plant collection and identification

*Balanites aegyptiaca*leaves, stem-bark and fruitwere collected from Gubi village(latitude 10^o 45′ N & longitude 9^o 82′ E) in Bauchi LGA, Bauchi State and was identified at the Herbarium Unit, Department of Biological Science, Ahmadu Bello University Zaria. A specimen voucher no: 900175was deposited.

3.1.2 Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma Aldrich, USA and BDH Ltd Poole, England. These include solvents; ethyl acetate, ethanol, diethyl ether, chloroform, and methanol. Chemicals include; Adenosine 5'-Triphosphate, Glucose-6-phosphate, β-NADP$^+$, β-NADH, Glycogen, Fructose 6-phosphate, Phosphoenolpyruvate, Frutose-1,6-bisphosphate, D-Glucose, Adenosine 5-diphosphate,Silica gel (60-200 mesh size), Metformin (Hovid Bhd, Malaysia)and Streptozotocin (Adooq Bioscience, LLC, United States).Enzymes include; Phosphoglucomutase, Glucose 6-phophate dehydrogenase, Pyruvate kinase, Lactate dehydrogenase.Reagent kit; Cholesterol assay kit (LOT No. 35020142), Triglycerides assay kit (LOT No.35040086), HDL-cholesterol assay kit (LOT No. 35030319) and Total protein assay kit (LOT No. 35020124) were of Agappe Diagnostics Switzerland GmbH. Albumin assay kit (REFAB362), Alanine aminotransferase (REF AL100) and Aspartate aminotransferase (REF AS101) kits were of Randox Lobaratories Limited, UK and Insulin ELISA kit (Mercodia, USA).

3.1.3 Equipment

Equipment used include; Spectrophotometer (Jenway6415 UV/Vis, England), Gas Chromatography-Mass Spectroscopy (Agilent Technologies, 7890B GC-System, 5977A MSD, USA), Fourier Transform Infra-Red (Agilent Technologies, Cary 630 FTIR, USA), Centrifuge-Clural (Labofuge 300, Heraeu), pH Meter (Agilent Technologies, 3200P, USA), Refrigerated Centrifuge (Z216 MK, Labnet International, Inc., USA), Weighing Scale (A.N.D GF-2000 Nettles Instrument, Switzerland), Glucometer (Accu-chek, Roche Diagnostics Co., Germany).

3.1.4 Experimental animals

A total of one hundred and sixty-four (164) wistar albino rats were used in the study;thirty-six (36) female rats were used for acute toxicity study, forty-eight (48) healthy rats of eithersex were used for preliminary studyof plant extract-fractions on normoglycemia and oral glucose tolerance. Whereas,eighty (80) diabetic-induced malerats were used to assess effects of the extract-fractions and subfractions. The rats were obtained from the Animal House,University of Jos, Plateau State and kept in clean cages with 12 hours / 12 hours light/dark photoperiod. Water and feed 'growers mash'(Vital feeds, Jos) were supplied *ad libitum*. The rats were allow to growweighting between 180-230g before used. Experimental protocol was in conformity with national and international laws and guidelines for care and use of laboratory animals as in **'**Principle of laboratory animal care' (NIH, 1985).

3. 2 Methods

3.2.1 Experimental design

The powdered form of the plant leaves, stem-bark and fruit-mesocarp was each extracted with ethanol followed by partitioning with water and ethyl acetate. Acute toxicity of each of the ethyl acetate and aqueous fractions was studiedin wistar albino rats followed by quantification of total phenolics and flavonoids content. Then, prelimenary study of theeffects of extract-fractions on normoglycemic and glucose tolerance in rats were conducted.

Diabetes mellitus was induced in rats by the injection of streptozotocin followed by the study of effects of the extract-fractions on oral glucose tolerance and thenthe antihyperglycemic effect. The diabetes mellitus study was divided into two sections. In the first section, the antihyperglycemic effect of the extract-fractions of *Balanites eagyptiaca* parts were investigated in the STZ-induced diabetic rats for 28 days period where biochemical parameters and enzymes like glucokinase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenol pyruvate carboxylkinase, glycogen phosphorylase, glycogen synthase and glucose-6-phosphodehydrogenase were assayed.

In the second section, the most potent extract-fraction of *Balanites aegyptiaca* part was further fractionated by gel filtration chromatograph. Then,antihyperglycemic effects of the subfractions obtained were evaluated in STZ-induced diabetic rats by same procedure as done in the first section. Thereafter, the content of the most potent subfraction was elucidated by FTIR and GC-MS. The extractswere administered orally using oral gastric tube. Rats were sacrified by decapitation at the end of each section of the experiments, blood and liver were collected. The liver homogenates were used for assayed of the glucose metabolizing enzymes while serum was used for biochemical parameters determinations.

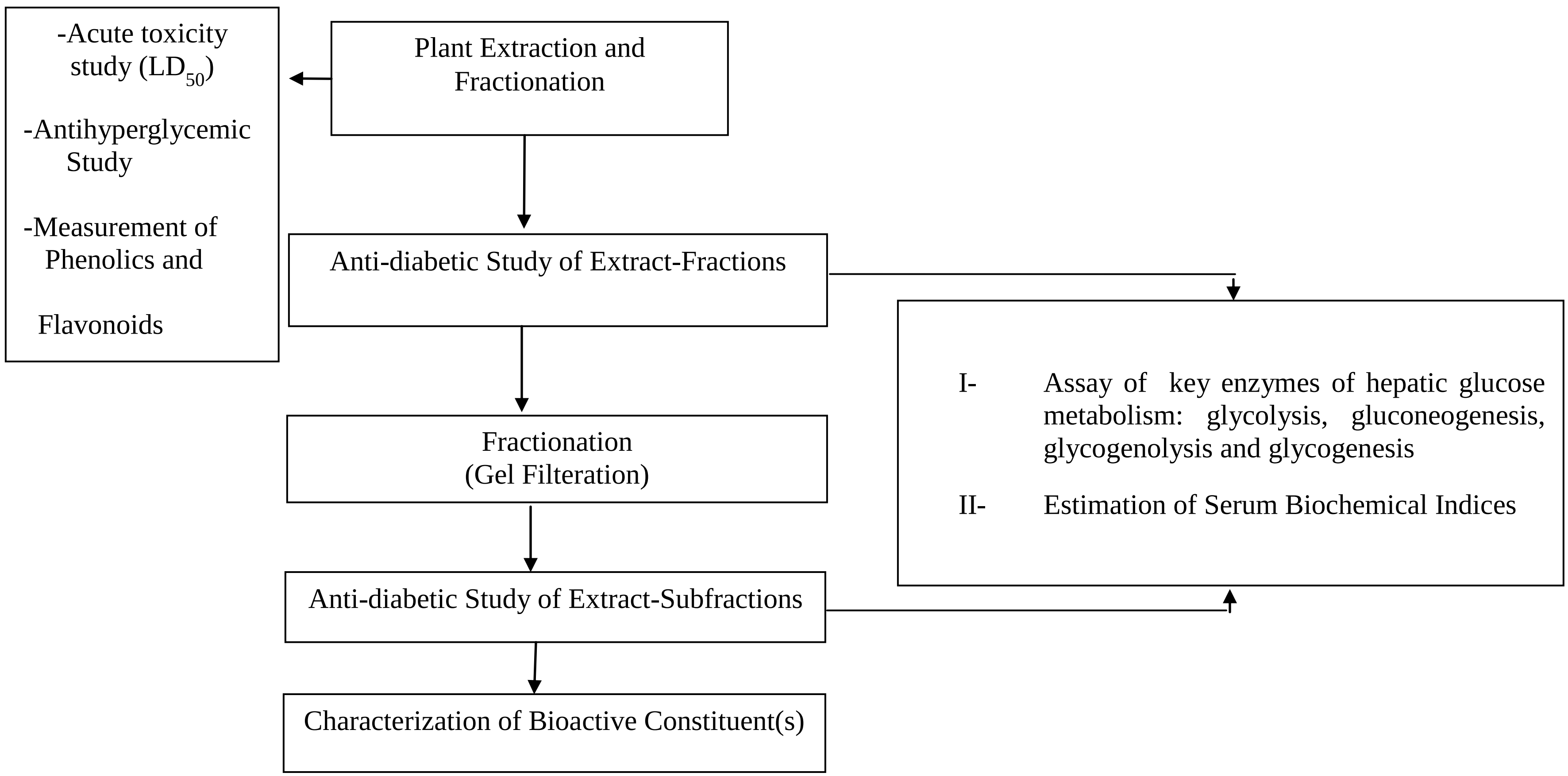


Figure 3.1 Flow chart of the experiment design to the study

3.2.2Plant sample defattening, extraction and fractionation

The plant leaves, fruit-mesocarp and stem-bark after collection were air-dried under shade for a week period then pulverized using pestle and mortar into powdered form. Plant samples were defatted as reported by Jung *et al* (2002) while the extraction and fractionation was performed as reported byGovorko *etal* (2007) with modification in choice of the extraction temperature (60 ^oC). Seven hundred and fifty gram (750g) powdered of each plant part was defatted for 2 hours with 1200 ml hexane on mechanical shaker. The hexane solvent was discarded, and the defatted samples were air-dried then exactly200 g each of the defatted plant leaves, stem-bark and fruit-mesocarp were mixed with 2000 mlof 80 % ethanol and heated to 60 °C for 2 hours. The extraction was continued for an additional 10 hrs at 20 °C. The mixture was filtered through a cheese cloth and resulting ethanol extract was air-dried. The procedure was repeated twice with same amount of defatted plant leaves, stem-bark and fruit-mesocarp.The ethanol extractof the leaves (183.44 g), stem-bark (156.42 g) and fruit-mesocarp (150.42 g) obtained weredissolved in water (500 ml) and partitioned with ethyl acetate (500 ml) at 20 ^oC for 2 hours then separated using a separating funnel (1000 ml). Each fraction was concentrated using a rotary evaporator at 40 ^oC and air dried. The dried extract-fractions of various parts of*Balanites aegyptiaca*were stored in air-tight containers and kept in a refrigerator at 4 ^0C until used.

3.2.3 Acute toxicity study

Acute toxicity study was performed according to guidelines of Organization of Economic Company and Development (OECD) 425 (1988). The extracts were administered in a single dose using oral gastric tube. Rats were deprived of food 3 hours prior to dosing. After each extract administration, observation was doneat 30 minutes interval for 4 hours then after 24 hours for behavioral change or death. The dosage; 5, 50, 500, 1000, 2000, 4000 mg/kg body wt were determined and used according to the OECD 425 guidelines with limit between the range of 2000-5000 mg/kg body wt.

Bruce (1985) up and down procedure for oral acute toxicity study was used to investigate acute toxicity in wistar albino rats. Briefly, rats were dosed one at a time and observed for survival or death. If a rat survives, the dose for the next rat was increased if it die the dose was decreased. Each rat was observed at least for 24 hours before dosing the next rat until the least dose lethal to rat is obtained.

Lethal Median Dose (LD_{50}) was calculated using the formula;

$$LD_{50} = (\text{the apparent least dose lethal to rats} - [(a \times b)/N])$$

Where, N = number of animal used, a = dose difference, and b = mortality.

Hence, therapeutic dose was calculated as $1/10^{th}$ of the lethal dose for the antidiabetic investigation.

3.2.4 Determination of total flavonoid content (TFC)

The total flavonoid content of aqueous and ethyl acetate fractions of *Balanitesaegyptiaca* leaves, stem-bark and fruit-mesocarp was determined using the methoddescribed by Meda *et al* (2005). Briefly, exact 1.0 ml (i.e. 1.0 ml contained 0.1 mg/ml) of aqueous and ethyl acetate fractions each was diluted with 4.0 ml of water in a 10 ml volumetric flask. Exact 3.0 ml of 5% $NaNO_2$ solution was added to each volumetric flask and allowed to stand at 25 ^oC for 5 min, then 0.3 ml of 10% $AlCl_3$ was added and allowed to standat 25 ^oC for 6 min. Exact2.0 ml of 1.0 M NaOH was added to stop the reaction. Finally, 2.4 ml of water was then added to the reaction flask and mixed. Absorbance of the mixture was read at 510 nm. TFC were determined as quecertin equivalents (mg/g of dry weight). The procedure were repeated trice for each sample and the results averaged.

3.2.5 Determination of total phenolics

Folin-Ciocalteu reagent was used to determine total phenolics content of the ethanol extract-fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp (Singleton *et al*., 1999). Briefly, about 1.0 ml of the ethyl acetate and aqueous fractions (0.1mg/ml) each was oxidized with 2.5 ml of 10 % Folin-ciocalteu reagent and neutralized by the addition of 2.0 ml of 75 g/L sodium carbonate. The reaction mixture was incubated for 40 min at 45 ^oC, then the absorbance was determined.A blank solution (without extract) was also run in similar way. Gallic acid was used as the standard compound to compare results. Five different concentrations of gallic acid in the range of 0.01 to 0.10 mg/ml was prepared and run by same procedure as done for the extract-fractions and the blank.

The absorbances of the sample and standard was readagainst the blank at 765 nm in the spectrophotometer. Unknown concentration of sample was evaluated using the gallic acid standard curve plotted. Total phenolics of the extract-fractions was determined by the formula below and result was expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/g).

Formula: $T = C \times V / M$

Where; T = Total phenolic contents in mg GAE/g of plant extract. C= the unknown concentration sample calculated from standard curve in mg/ml. V = the volume of the extract taken in ml and M = the weight of plant extract taken in grams.

3.2.7 Effect of ethanol extract-fractions on normoglycemic rats

Hypoglycemic effects of ethyl acetate and aqueous fractionsof *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp wereassessed on overnight fastednormoglycemic rats. Prior to the extract-fractions administration, blood was collected from the tail vein of each rats and glucose levelwas measured using a glucometer. Extract-fractions were then administered orally to the rats in each group using oral gastric tube. After half an hour intervals(i.e 30, 60, 90 and 120 min)blood glucose level wasmeasured from blood collected throught the tail vein of each rat by the used of a glucometer. Rats weredivided into 8 groups of 3 rats as follows:

Group 1:Rats received 10 % DMSO (Negative Control)

Group 2:Rats received Metformin at 200 mg/kg body wt. (Positive Control)

Group 3:Rats received aqueous leaves fraction (ALF) at 400 mg/kg body wt.

Group 4: Rats received ethyl acetate leaves fraction (ELF) at 400 mg/kg body wt.

Group 5: Rats received aqueous fruit-mesocarp fraction (AFF) at 400 mg/kg body wt.

Group 6: Rats received ethyl acetate fruit-mesocarp fraction (EFF) at 400 mg/kg body wt.

Group 7: Rats received aqueous stem-bark fraction (ASF) at 400 mg/kg bodywt.

Group 8: Rrats received ethyl acetate stem-bark fraction (ESF) at 400 mg/kg body wt.

3.2.7Effect of ethanol extract-fractionson gluocose loaded in normoglycemic rats

The effectsof ethyl acetate and aqueous fractions ofthe various parts of *Balanites aeygptiaca* were assessed onglucose tolerancein overnight-fasted normoglycemic rats. Prior to the extract-fractions administration, blood glucose levels were determined from blood collected through the tail vein of each rats by the used ofa glucometer. Extract-fractions were then administered orally to the fasting rats in each group using oral gastric tube. After half an hour interval, glucose solution (2 g/kg body weight) was fed by oral administration. Exactly 30 min after the glucose administration, blood glucose levels weremeasured through blood collected from the tail vein of each rat by the used of a glucometer. The procedure was repeated after 30 min interval for the next 60, 90 and 120 minutes. Rats were divided into 8 groups of 3 rats as follows:

Group 1: Rats received 10 % DMSO (Negative Control)

Group 2: Rats received Metformin at 200 mg/kg body wt. (Positive Control)

Group 3: Rats received aqueous leaves fraction (ALF) at 400 mg/kg body wt.

Group 4: Rats received ethyl acetate leaves fraction (ELF) at 400 mg/kg body wt.

Group 5: Rats received aqueous fruit-mesocarp fraction (AFF) at 400 mg/kg body wt.

Group 6: Rats received ethyl acetate fruit-mesocarp fraction (EFF) at 400 mg/kg body wt.

Group 7: Rats received aqueous stem-bark fraction (ASF) at 400 mg/kg body wt.

Group 8: Rats received ethyl acetate stem-bark fraction (ESF) at 400 mg/kg body wt.

3.2.8 Induction of diabetes mellitus (DM)

Type I diabetes mellitus was induced in rats by intra-peritoneal injection of 200 μLstreptozotocin (STZ) at a dose of 60 mg/kg body weight dissolved in 0.1 M citrate buffer (pH 4.5). Rats were given10 % glucose solution in their drinking water for 48 hours after STZ injection in order to prevent severe hypoglycemia. After 72 hours, blood glucose levels were checked and subsequently one week intervals to identify the onset and continued presence of diabetic hyperglycemia. Rats with fasting blood glucose level ≥200 mg/dl were considered diabetic and selected for the study (Gajidosik *et al*., 1999).

3.2.9Effects of ethanol extract-fractions on glucose tolerance in diabetic rats

The effects of ethyl acetate and aqueous fractions of various parts of *Balanites aeygptiaca* were assessed on glucose tolerancein overnight-fasted streptozotocin-induced diabetic rats. Prior to the extract-fractions administration, blood glucose levels weremeasured from blood collected through the tail vein of each diabetic rats used ofa glucometer. Extract-fractions were then administered orally to the diabetic rats groups using oral gastric tube. After half an hour interval, glucose solution (2 g/kg body weight) was fed by oral administration. Exactly 30 min after the glucose administration, blood glucose levels weremeasured from blood collected through the tail vein of each rat by the used of a glucometer. The procedure was repeated after 30 min interval for the next 60, 90 and 120 minutes.

Rats were divided into 8 groups of 3 rats as follows:

Group 1: Diabetic rats received 10 % DMSO (Negative Control)

Group 2: Diabetic rats received Metformin at 200 mg/kg body wt.

Group 3: Diabetic rats received aqueous leaves fraction (ALF) at 400 mg/kg body weight.

Group 4: Diabetic rats received ethyl acetate leaves fraction (ELF) at 400 mg/kg body weight.

Group 5: Diabetic rats received aqueous fruit-mesocarp fraction (AFF) at 400 mg/kg body weight.

Group 6: Diabetic rats received ethyl acetate fruit-mesocarp fraction (EFF) at 400 mg/kg body weight.

Group 7: Diabetic rats received aqueous stem-bark fraction (ASF) at 400 mg/kg body weight.

Group 8: Diabetic rats received ethyl acetate stem-bark fraction (ESF) at 400 mg/kg body weight.

3.2.10Effects of extract-fractionsof *Balanites aegyptiaca* parts in diabetic rats

The effects of ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp were assessed in streptozotocin-induced diabetic rats. Each extract-fraction at a dose of 400 mg/kg body weight was administered orally to rats groups using oral gastric tube. Rats were randomly allocated into nine (9) groups of 5 rats each, as follows:

Group A_I: Diabetic + aqueousleaves fraction (ALF)

Group A_{II}: Diabetic + ethyl acetate leaves fraction (ELF)

Group B_{I}: Diabetic + aqueous fruit-mesocarp fraction (AFF)

Group B_{II}: Diabetic + ethyl acetate fruit-mesocarp fraction (EFF)

Group C_{I}: Diabetic + aqueous stem-bark fraction (ASF)

Group C_{II}: Diabetic + ethyl acetate stem-bark fractions (ESF)

Group D: Diabetic + Metformin (200 mg/kg body wt)(Kolawole and Akanji (2014)

Group E: Diabetic control received 10 % DMSO

Group F: Normal control received 10 % DMSO

3.2.11Gel filtration chromatography

The potent extract-fraction (aqueous leaves fraction, ALF)based on the first section of the study was fractionated by Gel Filtration Chromatograph using silica gel (60-200 Mesh) packed in a chromatographic column while diethyl either, chloroform and methanol were used as the mobile phase. During the packing of the column, the lower part of the glass column was stocked with glass wool with the aid of glass rod. Exactly, 75 g of silica gel was dissolved in 180 ml of absolute chloroform to make the slurry. The chromatographic column was packed with silica gel and allowed free flow of the solvent into a conical flask. The set up was in order as the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed to stand for 24 hours to stabilize, the clear solvent at the top of the silica gel was drained down the silica gel meniscus.

Exact 5.0 g was dissolved in 5.0 mlof distilled water and then applied unto the chromatographic column. Elution of the extract was done with solvent system of increasing polarity: diethyl ether, diethyl ether/chloroform (1:1 v/v), chloroform and methanol. Exact400 mlof each solvent was poured into the column each time using Syringe. The eluted fractions were collected in aliquots of 40 ml in test tubes. The solvent in each tube was allowed to evaporate at room temperature (25 ^{o}C) to solid form.The whole procedure was repeated until about 150 g of the aqueous leaves fraction (ALF) was used.

3.2.12 Thin layer chromatography (TLC)

Thin layer chromatography of the subfractions was performed on silica gel G-coated

plates (0.25 mm for analytical). Exactly, 50.0 µl(100mg extract-subfraction dissolved in 1.0 ml distilled water) each of the diethyl ether, diethyl ether/chloroform, chloroform and methanol subfractions derived from ethanol-aqueous leaves fraction of*Balanites aegyptiaca*was saparately applied 1 cm above the lower edge of the thin layer chromatograph slide and dried. It was immersed to a depth of one cm in the solvents system: butanol, acetic acid and water in the ratio of 6:3:1. Compounds were visualized under UV light (254 nm).

3.2.13Effects of plant leaves extract-subfractionsin diabetic rats

Effect of diethyl ether, diethyl ether/chloroform, chloroform and methanol subfractions derived from ethanol-aqueous leaves fraction of *Balanites aegyptiaca*wereassessed on glucose metabolizing enzymes in streptozotocin-induced diabetic rats. Each subfraction at a dose of 400 mg/kg body weight was administered orally to rats using oral gastric tube. Rats were randomly allocated into four (4) groups of 5 rats each, as follows:

Group G: Diabetic + Diethyl ether leaves subfraction (L1)

Group H: Diabetic + Diethyl ether/chloroform leaves subfraction (L2)

Group I: Diabetic + Chloroform leaves subfraction (L3)

Group J: Diabetic + Methanol leaves subfraction (L4)

Group K: Diabetic + Metformin (200 mg/kg body wt) (Kolawole andAkanji (2014)

Group L: Diabetic control

Group M: Normal control

3.2.14Identification of bioactive compounds in*Balanites aegyptiaca*

The most potent sub-fraction derived from the ethanol-aqueous fraction of*Balanites aegyptiaca* part wassubjected to Infra red (IR) and Gas chromatograph-mass spectroscopy for elucidation of bioactive constituents. The compounds were identified using the database of the National Institute Standards and Technology (NIST) library.

3.2.14.1Fourier transformsinfra red spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used for identifying chemical bonds (functional groups) of compound present in bioactive sub-fractions of *Balanits aegyptiaca*leaves extract as described bySegneanu *et al* (2012). Dried powders of different sub-fractions were used for FTIR analysis during which 10 mg of the dried sub-fractionwas encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The extract was loaded in FTIR

Spectrophotometerwith a scan range from 1000 to 4000 cm^{-1} with a resolution of $4cm^{-1}$.

3.2.14.2 Gas chromatograph-massspectroscopy(GC-MS)

GC-MS analysis of the potent sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves was done by the method described by Clement and Taguchi (1989) using Shimadzu GC-MS equipped with silica type DB1 capillary column (30 m × 0.25 mm i.d.), film thickness 0.1 μm. The temperature of injector was 300 °C and the oven temperature was 300 °C. Helium (99.9%) was used as the carrier gas at a flow rate of 0.90 mL/min. Samples of 2 μL of *Balanites aegyptiaca*

were injected.

3. 3 Feed and Water Intake Estimations

During the experiment, food and water intake were recorded daily per each group. Feed was weighed using a weighing scale to ascertain the quantity given and the remnants in each rat groups after 24 hours. The volume of watergiven and after 24 hours was measured using a measuring cylinder (1000 ml).

3.4 Animal Body Weight and Pack Cell Volume Estimations

Animal weightwasdetermined weekly by weighing the rats using a weighing scale. The rats were weighed after an overnight fast by properly placing the rats in the weighing pan of the weighing scale thereafter theweights were recorded.

The percentage pack cell volume (PCV) of rats was determined by the method described by Schalm *et al* (1975). Blood was directly taken from the tail vein of rats into a plain capillary tube by capillary action to about three quarters of their lengths. One end of the tube was completely sealed using plasticine wax. The sealed capillary tubes were transfered to the micro-heamatocrit centrifuge placed in such a way that the sealed end pointed outward. The lib of the centrifuge was tightened to prevent blood spillage and then centrifuged for 5 minutes at 10,000 x g. Thereafter, the tubes with their content were transferred to the heamatocrit reader by placing in a position where the base of the blood in the column aligned with the 0 mark and then the packed cell volume was read in percentage (%).

3. 5Determination of Blood Glucose Levels (Beach and Turner 1958).

Principle: Glucose oxidase catalyses the oxidation of glucose to form glucuronic acid and H_2O_2. H_2O_2reacts with 4−aminoantipyrine and 4-chlorophenol in the presence of peroxidase to produce red chromogen which can be measured using spectrosphotometer.

Procedure: After an overnight fast,blood was collected from the tail vein of rats and was used to measure theirblood glucose levelsusing a glucometer. The total area under fasting blood glucose curve was determined using the formula described by

Tai (1994).n

$$\text{Area} = \frac{1}{2} \sum_{i=1}^{n} X_{i-1}(Y_{i-1} + Y_i)$$

(Tai formula)

3.6 Determination of Hepatic Glycogen Content

The isolation and hydrolysis of glycogen in the liver tissues was done by the method described by Good *et al* (1933).

Principle: When tissue is heated with strong alkali, glycogen is released which is precipitated upon addition of ethanol. Glycogen is then hydrolyzed in acid and glucose is released.

Isolation of glycogen: Liver tissue (1.0 g) was placed in 4.0 ml of KOH (30 %)then heated in boiling water for 10 min. After cooling, 0.2 ml of Na_2SO_4(20 %) and 5.0 ml of ethanol (95 %) were added and kept at 20 ^oC for 5 min. After precipitation was completed, the mixture was centrifuged at 3000 x g for 10 min and decanted. The packed glycogen in the tube was dissolved by addition of 5.0 ml distilled water with gentle warming.

Hydrolysis and estimation of glycogen: Exactly, 5.0 ml of HCl (1.2 mol/L) was added to 5.0 ml of the sample in test tubes then neutralized by the addition of 2 drops of 0.5 mol/L NaOH then cooled.Glucose in the sample was measured by the method described byCarroll*et al* (1956).A reagent blank was prepared by pipetting 1.0 ml of distilled water into a clean test tube. Exactly1.0 ml of sample and standard glucose solution (0.5 mg/ml of glucose)was pipetted into a clean separate tubes. Then, 5.0 ml of anthrone reagent was delivered into each tube and tightly capped. The test tubes were placed in a cold water bath. After all tubes have reached the temperature of the cold water, they were immersed in a boiling water bath to a depth little above the level of the liquid in the tubes for 15 minutes and was then removed and placed in a cold water bath and cooled to room temperature. Absorbances of test and standard were read against the reagent blank at 620 nm.

Calculation:

Glycogen (mg/g liver tissue) = $\frac{OD_u}{OD_s} \times 0.5 \times \frac{\text{Vol. of sample} \times 100}{\text{g of tissue}} \times 0.9$

Where,

OD_U = optical density of the unknown, OD_S = optical density of the standard, 0.5 = g of glucose in 0.5 ml of standard solution, 0.9 = factor for converting glucose value to glycogen value. Anthrone reagent (contained 0.05 % anthrone, 1% thiourea, and 72 % H_2S0_4solution).

3.7 Determination of Fructosamine

(Acharya and Manning, 1980; Armbruster, 1987)

Principle: Fructosamine react with phenylhydrazine to form phenylhydrazone adduct with maximum absorption at 350 nm.

Procedure: Exactly 0.2 ml of serum andstandard (dihydroxy acetone prepared in varied concentrations; 0.03, 0.06, 0.12, 0.23, 0.46, 0.92mmol/L was to plot the standard curve)was mixed with 1.0 ml of 9 g/L NaCl and incubated at 37 ^{o}C for 10 min. Then, 1.0 ml of phenylhydrazine was added and absorbance read at 350 nm after 10 min.

Calculation: Unknown fructosamine conc.(mmol/L) was extrapolated from 1,3-dihydroxylacetone calibrated curve (Appendix XVII).

3.8Assay of Hepatic Glucose Metabolic Enzymes Activity

1. Glucokinase activity (Goward, 1986)

Principle: Glucokinase catalyzes the phosphorylation of glucose to glucose 6-phosphate. Glucose-6-phosphate is converted to 6 phosphate-D-gluconateby reduction of β-$NADP^+$through a reaction with glucose-6-phosphate dehydrogenase. One unit of glucokinaseactivity is defined as 1µmol of D-glucose phosphorylated to glucose 6-phosphateper minat pH 9.0 and 30°Cassay condition.

Procedure:Exactly 2.80 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.10 ml enzyme (glucose-6-phosphate dehydrogenase) solutionwas added. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (60mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then the absorbance was read at 340 nm after 10 minutes.

Calculation**:**

$$\text{Units/mg protein} = \frac{(A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})\ (3) \times df}{(6.22)\ (0.1)}$$

Where, 6.22 = millimolar extinction coefficient of NADPH at 340 nm

3 = volume of mixture (ml)

0.1= Volume (in milliliters) of enzyme used

df = dilution factor

Reaction cocktail contain: 60 mM Tris HCl Buffer, , 20 mM $MgCl_2$, 110 mM KCl, 4 mM ATP, 12 mM D-glucose, G6PDH (10 U), 0.9mM β-$NADP^+$.

2. Gluose 6-phosphate dehydrogenase activity (Deutsch, 1989).

Principle: Glucose-6-phosphate dehydrogenase catalyzes the conversion of glucose-6-phosphate to 6 phosphate-D-gluconateby a reduction of β-$NADP^+$. The enzyme was assayedbased on the rate of formation of NADPH. One unit of glucose-6-phosphate dehydrogenase is defined as 1 nmol of NADPH formed under the assay condition.

Procedure: Exactly 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. The content were incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (50mM glycyglycine) was added to the blank test. The mixture were mixed by inversion then the absorbance was read at 340 nm after 10 minutes.

Calculation**:**

$$\text{Units/mg protein} = \frac{(A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})\ (3)(df)}{(6.22)\ (0.1)}$$

Where, 6.22 = Millimolar extinction coefficient of β-$NADP^{+}$ at 340 nm

0.1= Volume (in milliliters) of enzyme used

df = Dillution factor, and 3 = Total volume in milliters of assay

Reaction cocktail contain; 50 mM glycyglycine Buffer, pH 7.4 at 25 ^{o}C, 2mM D-G6P, 0.67 mM β-$NADP^{+}$ and 10 mM $MgCl_2$.

3. Phosphofructokinase (PFKinase) activity (Hengartner and Harris, 1975)

Principle: Phosphofructokinase catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. The enzyme was assayed based on the rate of reduction of β-NADH through a coupled reaction with pyruvate kinase and lactate dehydrogenase. One unit of Phosphofructokinaseactivity is defined as 1µmol of fructose-1,6-bisphosphateformed per min under the standard assay condition.

Procedure: Exact 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (100mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then incubated at 30 ^{o}C for 3 minutes and then the absorbance was read at 340 nm.

Calculation**:**

$$\text{Units/mg protein} = \frac{(A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank}) \times 3 \times df}{(6.22)\ 0.1}$$

Where, 6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

0.1= Volume (in milliliters) of enzyme used

df = Dillution factor, 3 = Total volume in milliters of assay

Reaction cocktail contain: 100 mM Tris-HClbuffer(pH 9.0 at 30^{o}C), 500mM D-fructose-6-phosphate, 13.1 mM NADH and 10 mM $MgSO_{4,}$ PK/LDH (5 U),0.035 M ATP, 2.5 M KCl.

4. Fructose-1,6-bisphosphatase activity (Majumder and Eisenberg, 1977)

Principle: Fructose-1,6-bisphosphatase catalyzes the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. The enzyme activity was assayedby the measuring of the amount of inorganic phosphate liberated.The enzyme activity unit is defined as 1µmol of phosphate liberated per minute under standardassay condition.

Procedure: Exact 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exact, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (100mM Tris HCl) was added to the blank test. The mixture were mixed by inversion and incubated at 37 ^{o}C for 1 hour. Then, 1.0 ml of trichloroacetic acid (15%) was added and centrifuged at 10,000 x g. Exact 1.5 ml of the supernatant was mixed with 1.5 ml of ammonium molybdate (2 g ammonium molybdate was dissolved in 5.55 ml conc. H_2SO_4 and was made to 100 ml with H_2O). After about 10 mins, absorbance of ammonium phosphomolybdate complex formed was read at 840 nm in a Spectrophotometer(Hansjorg and William, 1969).

Calculations:

$$\text{Units/}\mu\text{mole } p_i \text{ liberated} = \frac{(A_{840nm}\text{ Test} - A_{840nm}\text{ Blank})(3)}{(3.0*0.3)}$$

Where, 3 = Total volume (in milliliters) of assay

3.0 = Millimolar extinction coefficient of inorganic phosphate at 840 nm

Reaction cocktail: 100 mM Tris-HCl buffer (pH 8.0); 10 mM $MgCl_2$, 1 mM EDTA and 1 mM fructose-1, 6-bisphosphate.

5. Phosphoenolpyruvate carboxylkinase activity (Berndt and Ulbrich 1970).

Principle: Phosphoenolpyruvate carboxylkinase catalyzes the conversion of oxaloacetate to phosphoenol pyruvate coupled by its conversion to pyruvate and lactate in the presence of β-NADH through reactions with pyruvate kinase and lactate dehydrogenase. Enzyme activity unit was expressed as 1 µmol phosphoenol pyruvateformedper minute under standard assay condition..

Procedure: Exactly 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (100 µmoles Tris HCl) was added to the blank test. Themixture was mixed and incubated at 37 °C for 15 min., then 0.1 ml of TCA (3 M) was added to the content in both tubes. The mixture was centrifuged at 1000 x g for 10 min and 1.0 ml of 2,4-dinitrophenylhydrazine (0.3 % in 2 N HCl) was added to the supernatant. After about 20 min, 5.0 ml of ethyl acetate and water (1:1 v/v) was added. The ethyl acetate portion was decanded and 1.0 ml of the aqueous portion was neutralized with 0.10 ml of 3.5 N NaOH followed by the addition of 1.0 ml solution of 0.15 M NADH, pyruvate kinase (5 U) and lactic dehydrogenase (5 U). The mixture was incubated at 25 ^{o}C for 5 min then the absorbance was read at 340 nm.

Calculations:

$$\text{Units/mg protein} = (A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})\ (3)/\ (6.22*0.1)$$

Where, 3 = Total volume (in milliliters) of assay

6.22 = Millimolar extinction coefficient of NAD^+ at 340 nm

Reaction cocktail: 100 µmoles Tris buffer (pH 8.0), 1 µmoles $MnCl_2$, 20 µmoles $MgSO_4$, 10 µmoles KCl, 10 µmoles KF, 7 µmoles ATP and 3µmoles of oxaloacetate.

6. Glycogen phosphorylase activity (Morgan and Parmeggiani, 1964)

Principle:Glycogen phosphorylase degradates glycogento glucose-1-phosphatecoupled with its conversion toglucose-6-phosphate by phosphoglucomutase. Glycogen phosphorylase is assaythrough the conversion of glucose-6-phosphate to 6-phosphate-D-gluconateby a reduction of β-$NADP^+$ with glucose-6-phosphate dehydrogenase. One unit of enzyme activity is defined as 1µmol of glucose-1-phosphateformed per min under the assay condition.

Procedure: Exactly 2.88 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.01 ml each of glucose-6-phosphate dehydrogenase solution and phosphoglucomutase solutionwere added to the content in both tubes. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (20mM Imidazole) was added to the blank test. The mixture were mixed by inversion and was allow to stand for 10 minutes at 25 ^{o}C then the absorbance was read at 340 nm.

Calculations:

$$\text{Units/mg protein} = (A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})(3)(df)/\ (6.22)(0.1)$$

Where, 3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm

0.1 = Volume (in milliliter) of enzyme

Reaction cocktail: 20 mM imidazole (pH 7, 30 ^{0}C), 0.01 % bovine serum albumin, 150 mM potassium acetate, 3 mM glycogen, 20 mM magnesium chloride, 15 mM cysteine, 10 mM inorganic phosphate, 0.1 mM ß-NADP^{+}, 20 µg glucose-6-phosphate dehydrogenase (10 U) 40 µg phosphoglucomutase.

7. Glucose-6-phosphatase (G6Pase) activity (Baginski *et al*., 1974)

Principle: Glucose-6-phosphatase catalyzes the conversion of glucose-6-phosphate to glucose and inorganic phosphate. The enzyme activity was assayedby the measuring of the amount of inorganic phosphate liberated.

Procedure: Exactly 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (135mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then incubated at 37 ^{o}C for 1 hour. Exactly 1.0 ml of trichloroacetic acid (15%) was added and centrifuged at 10,000 x g. Exactly 1.5 ml of the supernatant was mixed with 1.5 ml of ammonium molybdate (2 g ammonium molybdate was dissolved in 5.55 ml conc. H_2SO_4 and was made to 100 ml with H_2O). The mixture were allow to stand for 10 minutes at 25 ^{o}C then the absorbance of ammonium phosphomolybdate complex formed was read at 840 nm in a Spectrophotometer(Hansjorg and William, 1969).

Calculations:

$$\text{Units/µmole } p_i \text{ liberated} = \frac{(A_{840nm}\text{ Test} - A_{840nm}\text{ Blank})(3)}{(3.0*0.3)}$$

The enzyme activity unit is defined as 1µmol of phosphate liberated per minute under standard assay condition.

Where, 3 = Total volume (in milliliters) of assay

3.0 = Millimolar extinction coefficient of inorganic phosphate at 840 nm

Reaction curtail contained: Tris-HCl bufer, 135 mM (pH 6.5), 5 mM EDTA, and 20 mM Glc-6-P.

8. Lactate Dehydrogenase (LDH) (Pesce, 1989).

Principle: Lactate Dehydrogenase catalyzes the reduction of pyruvate in the presence of β-NADH to lactate and β-NAD^+. One unit of enzyme activity is defined as1 µmol of β-NAD^+ formed in 1 min under the standard assay condition.

Procedure: Exactly 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (50mM Imidazole HCl) was added to the blank test. The mixture was mixed by inversion and incubated at 25 ^oC for 5 minutes then the absorbance was read at 340 nm.

Calculation**:**

$$\text{Units/mg enzyme} = \frac{(A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})\text{x}3\text{xdf}}{(6.22) \times 0.1}$$

Where, 3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of ß-NAD^+ at 340 nm

0.1 = Volume (in milliliter) of enzyme

Reaction cocktail: 50 mM Imidazole HCl buffer (pH 7.6 at 25 ^oC), 0.12 M KCl and 0.062 M $MgSO_4$, 6.6 mM NADH, 45 mM Pyruvate.

9. Glycogen synthase activity (Danforth, 1965)

Principle: Glycogen synthase catalyzes the conversion of uridinyldiphosphate-glucose in the presence of glycogen to uridinyldiphosphate which reacts with phosphenol pyruvate to pyruvate followed by a reduction of pyruvate in the presence of β-NADH to lactate and β-$NAD^+$by pyruvate kinase and lactate dehydrogenase. One unit of enzyme activity is defined as 1 µmole of glucose incorperated into glycogen from UDP-glucose under standard condition.

Procedure (phase I): Exactly 3.00 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, exactly 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (48mM Tris HCl) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^{o}C for 5 minutes then the reaction was stoped by placing the tubes in boiling water bath. It was cooled with running tap water and centrifuged.

Phase II: Exactly 2.80 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.01 ml enzymes (pyruvate kinase and lactate dehydrogenase) solutionwas added. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernant from the sample test reaction content in phase I) was added to the content in the test tube laballed as sample test whereas 0.10.ml of supernant from the blank reaction content in phase I) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^{o}C for 10 minutes then the absorbance was read at 340 nm.

Calculations: Units/mg protein = $\frac{(A_{340nm}\text{ Test - }A_{340nm}\text{ Blank})(2.91)}{5(6.22)\ (0.1)}$

Where, 2.91 = Total volume (in milliliters) of assay, 6.22 = Millimolar extinction

Coefficient of β-NAD$^+$, 0.1 = Volume (in milliliter) of enzyme, 5 = Time of Reaction (in minutes) of Step 1.

Reaction Cocktail: Step I(48 mM Tris-HCl, pH 8.2 at 30 ^oC, 12.4 mM $MgCl_2$, 1.0 mM EDTA, 1% glycogen, 3.63 mM UDP-glucose). Step II (48 mM Tris-HCl, pH 7.8, 25 mM potassium chloride, 1.0mM EDTA, 60 mM $MgSO_4$, 5.2 mM PEP, PK/LDH (5 U) 6.6 mM NADH).

10. Pyruvate kinase (PK) (Pogson and Denton, 1967).
Principle: Pyruvate kinase catalyzes the conversion of phosphenol pyruvate to pyruvate followed by a reduction of pyruvate in the presence of β-NADH to lactate and β-NAD$^+$. One unit of enzyme activity is defined as 1 µmol of phosphenol pyruvate converted to pyruvate in 1 minute under the standard assay condition.

Procedure: Exactly 2.89 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.10 ml enzyme (lactate dehydrogenase) solutionwas added. Exactly, 0.10 ml sample (supernant of the liver homogenate) was added to the content in the test tube laballed as sample test whereas 0.10.ml buffer (72mM ImidazoleHCl) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^oC for 10 minutes then the absorbance was read at 340 nm.

Calculation**:**

$$\text{Units/mg protein} = \frac{(A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})\text{x3xdf}}{6.22\text{x}0.1}$$

Where, 6.22 = Millimolar extinction coefficient of β-NAD$^+$ at 340 nm.

3 = Total volume (in milliliters) of assay

df = Dilution factor

0.1 = Volume (in milliliter) of enzyme

Reaction cocktail contain; 72 mM Imidazole HCl buffer, pH 7.2, 71.2mM KCl, 15.2mM $MgCl_2$, 5.2 mM PEP, 0.19 mM NADH, 7.6 mM ADP, 71.2 mM KCl, LDH (20U).

3.9Liver Function Test

I. Total Protein (Bradford, 1976)

Principle: The principle is based on the formation of blue colored complex when protein reactswith commassie dye under acidic condition. The protein is measured spectrophotometrically at 595 nm.

Procedure: Exactly 100 µl of sample (serum), standard solution, and distilled water were each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 3000 µl of Bradford reagent was added to the content in all the test tubes. The mixture was mixed by inversion and then incubated at 25 ^{o}C for 30 minutes. Then,the absorbance of the test and stardard were read against the blank at 595 nm.

Calculation: Total Protein (mg/g liver) = Abs. of sample/standard x 6000mg

II. Serum Albumin (Doumas *et al*., 1971)

Principle:The measurement of serum albumin is based on its quantitative binding

3,3’,5,5’-tetrabromom cresol sulphonephthalein (BCG). The albumin-BCG complex absorbs maximally at 630 nm, the absorbance is directly proportional to the concentration of the albumin in the sample.

Procedure: Exactly 0.01 ml of sample (serum), standard solution, and distilled water were each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 3.0 ml of BCG reagent was added to the content in all the test tubes. The mixture was mixed by inversion and then incubated at 25 ^{o}C for 5 minutes. Then,the absorbance of the test and stardard were read against the blank at 630 nm.

NB: BCG reagent: Bromocresol green (75 mmol/L, pH 4.2)

Calculation: Albumin (g/dl) = Abs. of sample/standard x 4.59 g/dl

III. Assay of aspartate and alanine aminotransferases activity
(Reitman and Frankel, 1975)

Principle:

1. α-oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + Oxoloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhyrazineand measured at 546 nm.

2. α-oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + pyruvate

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhyrazineand measured at 546 nm.

Procedure: Exactly 0.5 ml of test reagent 1 was pipetted into a clean separate test tubes labelled as sample test and blank test. Then, 0.1 ml of sample (serum) was pipetted and added to the content in the tube labelled as sample test whereas 0.1 ml of distilled water was added to the blank test. The mixture was mixed by inversion and then incubated at 37 ^{o}C for 30 minutes. Then,0.5 ml of reagent II was pipetted and added to the content in both tubes followed by the addition of 0.1 ml of distilled water to the content in the tube labelled as sample test whereas 0.1 ml of sample (serum) was added to the blank test. The mixture was mixed by inversion and then incubated at 25 ^{o}C for 20 minutes. Then,5.0 ml of NaOH solution was added to the content in both tubes, mixed and allow to stand for 5 miuntes then the the absorbance of testwas read against the blank at 546 nm.

Calculation: AST and ALT activity are extrapolated from the standard curve.

NB: AST Reagent 1 contained phosphate, L-aspartate and alphaoxaglutarate (100mmol/L). ALT Reagent 1 contanined phosphate, L-alanine and α-oxaglutarate (100mmol/L), Reagent 2 contained 2,4-dinitrophenylhyrazine (2 mmol/L).

3. 10Determinationsof Serum Lipid Profile

I. Determination of Serum triglyceride (TG)(Fossati and Prenape,1982)

Principle: This is based on the enzymatic hydrolysis of serum or plasma triglyceride

ride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase to form glycerol− 3−phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is then oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). The H_2O_2

reacts with 4‒ aminoantipyrine and phenol in the presence of peroxidase to produce red chromogen measured spectrophotometrically at 546 nm.

Procedure: Exactly 1000 µl of TG reagent was pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 10 µl of sample (serum) was added to the content in the test tube labelled as sample test, 10 µl of standard solution was added to the content in the standard test whereas 10 µl of distilled water was added to the content in the blank test tube. The mixture was mixed by inversion and then incubated at 37^{o}C for 5 minutes. Then,the absorbance of the test and stardard were read against the blank at 546 nm.

Calculation: Triglycerides conc (mg/dL) = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x 200 mg/dL

NB: TG reagent contained potassium ferrocynate 10 mmol/L, magnesium salt 17 mmol/L, 4-aminoantipyrine 0.9mmol/L, ATP 3.15 mmol/L, lipoprotein lipase >1800 U/L, glycerol kinase >450 U/L, glycerol-3-phosphate oxidase U/L, peroxidase >450U/L all in pipes-buffer pH 7.0.

II. Determination Total Cholesterol (Roeschlau *et al*., 1974)

Principle: Cholesterol esters present in a specimen are hydrolyzed into free cholesterol and free fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol is then oxidized by cholesterol oxidaseto cholesten -4-ene-3-one and hydrogen peroxide (H_2O_2). The H_2O_2 react with 4‒ chlorophenol and 4‒ aminoantipyrine in the presence of peroxidase to form quinoneimine dyes.

Procedure: Exactly 1000 µl of Chloresterol reagent was pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 10 µl of sample (serum) was added to the content in the test tube labelled as sample test, 10 µl of standard solution was added to the content in the standard test whereas 10 µl of distilled water was added to the content in the blank test tube. The mixture was mixed by inversion and then incubated at 37 ^{o}C for 5 minutes. Then,the absorbance of the test and stardard were read against the blank at 505 nm.

Calculation: Cholesterol conc. (mg/dL) = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x 200 mg/dL

III. Determination of High Density Lipoprotein Cholesterol (HDL-C) (Lopes-Virella *et al*., 1977)

Principle: The chylomicrons, VLDL and LDL of serum are precipitated by phosphotungstic acid and magnesium ions. After centrifugation HDL are in the supernatant. HDL content of supernatant is measured by an enzymatic method.

Procedure: Precipitation, exactly 300 µl of serum was mixed with 300 µl of HDL-reagent and was allowed to stand for 10 minat 25 ^{o}C. It was centrifuged at 4000 x g for 10 min then the supernatant was collected after an hour. The supernatant was used for the determination of the HDL-cholesterol. Exactly 1000 µl of chloresterol reagent was pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 50 µl of sample (supernant) was added to the content in the test tube labelled as sample test, 50 µl of standard solution was added to the content in the standard test whereas 50 µl of distilled water was added to the content in the blank test tube. The mixture was mixed by inversion and then incubated at 37 ^{o}C for 5 minutes. Then,the absorbance of the test and stardard were read against the blank at 505 nm.

Calculation: HDL Cholesterol conc. (mg/dL) = $\frac{\text{Abs of sample}}{\text{Abs of standard}}$ x 50 mg/dL x2

NB: Cholesterol reagent contained phosphotungstate 14 mmol/L & $MgCl_2$ 1mmol/L

IV. LDL Cholesterol and VLD L Cholesterol (VLDL-C) (Marchell,1992)

LDL – Cholesterol conc. (mg/dL) = [TC - (HDL-C + Triglycerides /5)]

VLDL-Cholesterol conc. (mg/dL) = [Triglycerides /5].

3. 11 Determination of Serum Insulin Levels

Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) method as desricbed by Clark and Hales (1994).

Principle: This was based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinations on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtitration well. After washing, unbound enzyme labeled antibody was removed. The bound conjugated insulin was detected by reacting with 3’,3’,5’,5’-tetramethylbenzidine and optical density measured with microplate autoreader at 450 nm.The intensity of the colour generated is directly proportional to the amountof insulin in the sample.

Procedure: Exactly 50 µl of insulin calibrators, control and samples were pipetted into separate wells then 100 µl of the insulin enzyme reagent was added to each well. The microplate was swirl gently for 30 seconds and was incubated for 120 minutes at 25 ^{o}C after which the content of the microplate was discarded. Then, exactly 350 µl wash buffer was added and decanded trice followed by the addition of 100 µl of the working substrate solution to all wells and incubated for 15 minutes. Finally, exact 50 µl of a stop solution was added and mixed gently for 20 seconds. Then, the absorbance of content in each well was read at 450 nm using a microplate reader.

Calculation: The concentrations of insulin (ng/L) in sample was ascertained from dose response curve for standard insulin (appendix XVII)

NB: Insulin enzyme reagent (enzyme labelled affinity purified monoclonal mouse x-insulin IgG, biotinylated monoclonal mouse x-insulin IgG in buffer, dye and preservative), wash solution (a suefactant in buffered saline), substrate A (tetramethlbenzidine in buffer), substrate B (hydrogen peroxidase in buffer), and stop solution (1N HCl).

3. 12Statistical Analysis

Data from the experiments were expressed as mean ± standard deviation (SD). Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan's multiple range test (DMRT) (Duncan 1957). Significant difference was accepted at $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1Yield and Phytochemical Constituents of Plant Extracts and Fractions

Thepercent yield of ethanol extracts of *Balanites aegyptiaca* parts are as follows; leaves extract 183.44 g (30.57 %), stem-bark extract 156.42 g (26.07 %) and fruit-mesocarp extract150.42 g (25.07 %). Whereas, the yield of ethanol-extract fractions of*Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp namely; aqueous leaves fraction (ALF), ethyl acetate leaves fraction (ELF), aqueous fruit-mesocarp fraction (AFF), ethyl acetate fruit-mesocarp fraction (EFF), aqueous stem-bark fraction (ASF), and ethyl acetate stem-bark fraction (ESF) is presented in Table 4.1.

The result of the phytochemical screeming of aqueous leaves fraction (ALF), ethyl acetate leaves fraction (ELF), aqueous fruit-mesocarp fraction (AFF), ethyl acetate fruit-mesocarp fraction (EFF), aqueous stem-bark fraction (ASF), and ethyl acetate stem-bark fraction (ESF)of *Balanites aegyptiaca*parts are also presented in Table 4.1.

4.2 Oral Acute Toxicity of Ethanol Extract-Fractions

Results of acute toxicity of ethanol extract-fractions showed no death or treatment-related mortality at all the tested doses, no significant changes in behavior such as apathy, hyperactivity and morbidity in the treated rats (Table 4.2). The extract-fractions of *Balanites aegyptiaca* leaves, stem-bark, and fruit-mesocarp are safe up to a dose of 4000 mg/kg body weight. The LD_{50} value for acute toxicity of extract-fractions of *Balanites aegyptiaca* parts is considered to be greater than 4000 mg/kg body wt.

Table 4.1 Yield and Phytochemical Constituents of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp

	Ethanol Extract-Fractions					
Chemical Constituents	**Aqueous LeavesFraction**	**Ethyl acetate LeavesFraction**	**Aqueous Fruit-mesocarp Fraction**	**Ethyl acetate Fruit-mesocarp Fraction**	**Aqueous Stem-bark Fraction**	**Ethyl acetate Stem-bark Fraction**
Fraction Yield (g/750 g)	126.22	57.12	130.80	18.96	132.24	20.62
Saponins	+	+	+	+	+	+
Terpeniods	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Tannins	+	+	+	+	+	-

+ = present, - = absent

Table 4.2. Acute Toxicity of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-bark andFruit-mesocarp on Normal Wistar Albino Rats

Extracts' Doses (mg/kg body wt.)	**Aqueous Leaves Fraction**	**Ethyl acetate LeavesFraction**	**Aqueous Fruit-mesocarp Fraction**	**Ethyl acetate Fruit-mesocarp Fraction**	**Aqueous Stem-bark Fraction**	**Ethyl Stem-bark Fraction**	**Inference**
5	- *	- *	- *	- *	- *	- *	Safe
50	- *	- *	- *	- *	- *	- *	Safe
500	- *	- *	- *	- *	- *	- *	Safe
1000	- *	- *	- *	- *	- *	- *	Safe
2000	- *	- *	- *	- *	- *	- *	Safe
4000	- *	- *	- *	- *	- *	- *	Safe

- = no mortality, * = no behavior change (Behavior changes: apathy, hyperactivity, morbidity)

4.3 Phenolics and Flavonoids Content of Various Parts of *Balanites aegyptiaca* EthanolExtract-Fractions

Total phenolics and flavonoids content of ethanol extract-fractions of Balanites aegyptiaca leaves, fruit-mesocarp and stem-bark are presented in Table 4.3. Phenolics and flavonoids content varied in the ethanol extract-fractions of *Balanites aegyptiaca* parts. Aqueous fruit-mesocarp fraction had the highest total phenolics ($77.74 \pm 1.68 x 10^{-2}$mg g^{-1} Garlic acid Equivalent) whereasaqueous stem-bark fraction has the lowest (51.51 ± 4.46 $x10^{-2}$mg g^{-1} Garlic acid Equivalent). Ethyl acetate stem-bark fraction has the highest amount of total flavonoids ($11.41 \pm 1.24 x 10^{-2}$mg g^{-1}Quercetin Equivalent) whereas, ethyl acetate leaves fraction had lowest ($1.48 \pm 0.01 x 10^{-2}$ mg g^{-1} Quercetin Equivalent). In general, phenolics content was high in the plant extracts compared to flavonoids.

4.4 Effect of Ethanol Extract-Fractions on normoglycemic rats

The hypoglycemic effects of ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp in normoglycemic rats are presented in Figure 4.1. The extract-fractions lowered blood glucose levels in a time depended manner. Decrease in blood glucose levels of rats that received metformin and aqueous fruit fraction (AFF) were observed after 30 minutes and continuedto 120 mins. Ethyl acetate fractions of leaves, fruit-mesocarp and stem-bark ethanol extracts showed less effect on the blood glucose levels. Reduction in blood glucose levels in rats groups that received aqueous-fractions of various parts of *Balanites aegyptiaca* and metformin were still within the normal range.

Table 4.3Total Phenolics and Flavonoids Content of Ethanol Extract-Fractions of*Balanites aegyptiaca* Leaves,Stem-Bark and Fruit-Mesocarp

	Ethanol Extract-Fractions					
	Aqueous LeavesFraction	**Ethyl acetate LeavesFraction**	**Aqueous Fruit-mesocarp Fraction**	**Ethyl acetate Fruit-mesocarp Fraction**	**Aqueous Stem-bark Fraction**	**Ethyl acetate Stem-bark Fraction**
Phenolics (mg g^{-1}GAE) x10^{-2}	66.08± 6.07^{bc}	56.37± 4.45^{b}	77.74± 1.68^{bcd}	6.51± 1.98^{a}	51.51± 4.46^{b}	6.32 ± 0.73^{a}
Flavonoids (mg g^{-1}QE) x10^{-2}	3.45± 0.04^{bc}	1.48± 0.01^{a}	2.58± 0.01^{b}	5.36± 0.01^{bcd}	3.59± 0.32^{bc}	11.41± 1.24^{bcde}

Values are Mean ± SD of triplicate determinantions.Values with different superscript across the rows are significantly different ($P<0.05$)

GAE = Garlic acid Equivalent, QE= Quercetin Equivalent

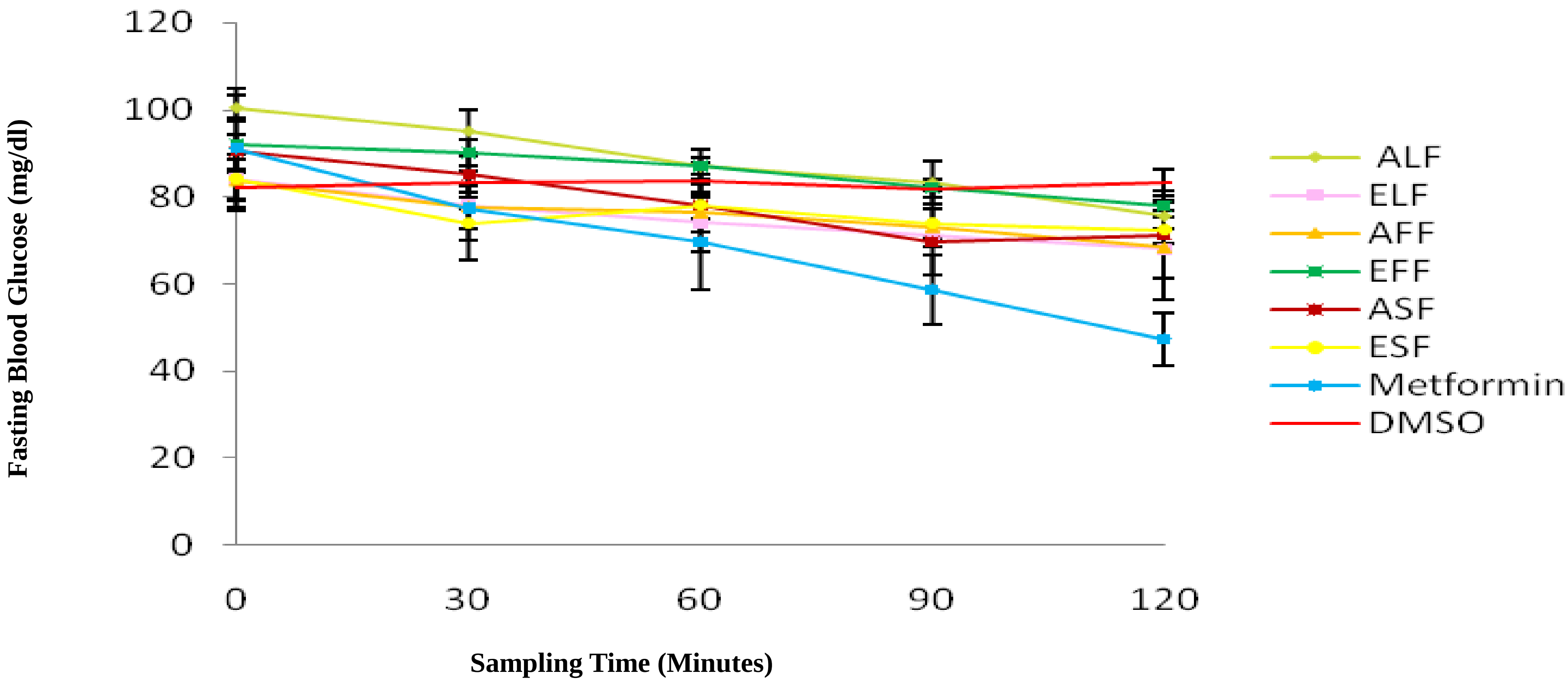


Figure 4.1Hypoglycemic Effect of Oral Administration of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Stem-Bark andFruit-Mesocarp in Normoglycemic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate Fruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

4.5Effect of Extract-Fractions on Glucose Tolerance in Normoglycemic Rats

Effects of oral administration of ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp on oral glucose tolerance in normoglycemic rats are presented in Figure 4.2. Postprandial blood glucose of rats that received metformin and aqueous leaves, stem-bark and fruit fractionswere suppressedcompared to the ethyl acetate fractions of leaves, fruit-mesocarp and stem-bark. Data of the study showed aqueous fraction of the plant parts suppressed posprandial blood glucose.

4.6Effect of Extract-Fractions of *Balanites aegpytiaca*on Oral Glucose Tolerance in Diabetic Rats

Blood glucose levels of various groups of STZ-induced diabetic rats treated with ethanol extract-fractionsof *Balanites aegyptiaca* leaves, fruit-mesocarp and stem-bark at 400 mg/kg body weight after oral administration of glucose is shown in Figure 4.3.A raise in blood glucose level was observed in the diabetic control rats after 30 min and remained high over the next 120 min. Raise in blood glucose levels in rats that received metformin, aqueous (ALF) and ethyl acetate (ELF) leaves fractions were suppressed after 60 min and remained low over the next 120 min. Ethyl acetate fruit-mesocarp (EFF) and stem-bark (ESF) fractions suppressed blood glucose after 90 min through 120 min. However, fluctuation in the blood glucose level were observed in rats that received aqueous fruit-mesocarp (AFF) and stem-bark (ASF) fractions.

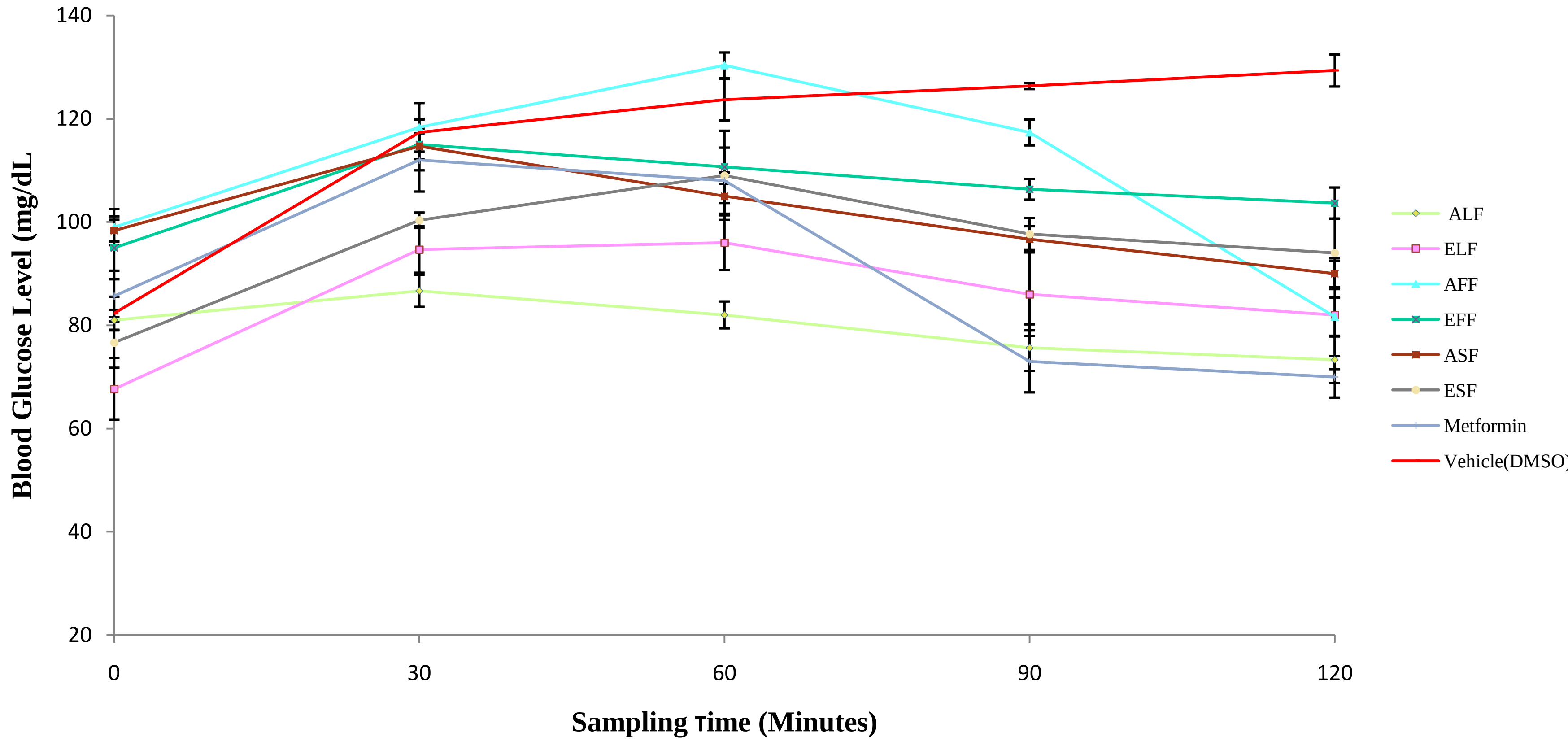


Figure 4.2Effect of Oral Administration of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Oral Glucose Tolerance in Normoglycemic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

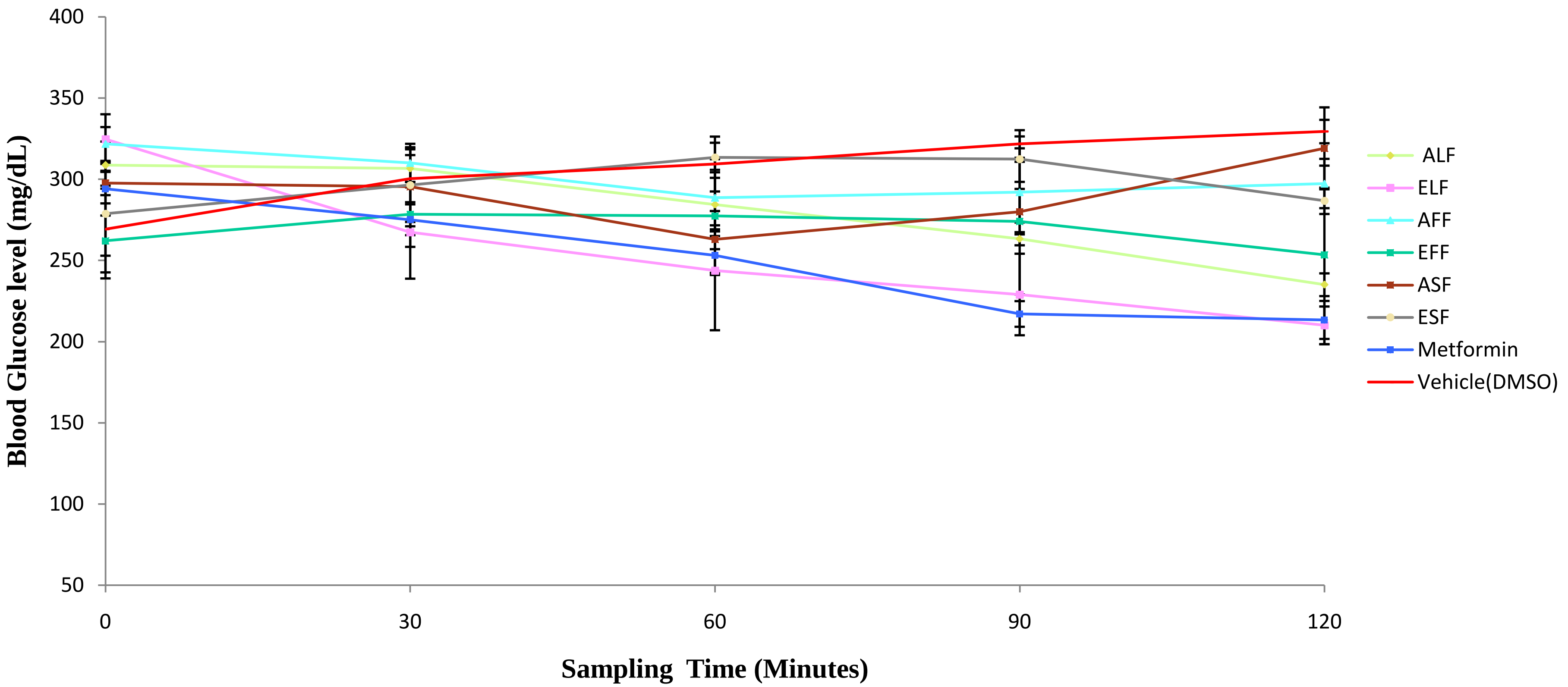


Figure 4.3Effect of Oral Administration of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Oral Glucose Tolerance in Streptozotocin-induced Diabetic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

4.7 Effects of Extract-Fractions of *Balanites aegpytiaca*Parts in Diabetic Rats

4.7.1 Anti-hyperglycemic effect of ethanol extract-fractions of *Balanites aegpytiaca* instreptozotocin-induced diabetic rats

The effect of various ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarpon blood glucose level in STZ-induced diabetic rats is givenin Figure 4.4.Diabetes mellitus is characterized by hyperglycemia, in the diabetic untreated rats,levels of fasting blood glucose gradually raised and continued throughout the experiment period (246.80±7.46 – 336.69±11.91 mg/dl). Decreased fasting blood glucose level indiabetic rats that received metformin and plant extract-fractions were recorded as the treatment duration advancesand were significantly different($P<0.05$) compared to the diabetic untreated group. Diabetic rats treated with metformin had lowered fasting blood glucose by 24.62 % followed by the rats group that received aqueous fruit-mesocarp fraction (AFF) 18.61 % and aqueous leavesfraction (ALF) 15.87 % whereasother extract-fractions had less than 15.00 % reuction in fasting blood glucose.Aqueous fruit-mesocarp (AFF) and aqueous leaves (ALF) fractions of *Balanites aeypytiaca*were the most potentbased on percent fasting blood glucose reduction as well as their low value of area under the curve(Figure 4.5).

4.7.2Effect of extract-fractions of *Balanites aegpytiaca*on serum insulin level in streptozotocin-induced diabetic rats

Increasedin serum insulin levels were recorded in diabetic rats following oral administration of ethyl acetate and aqueous fractions of *Balanites aegyptiaca* parts; aqueous leaves fraction (0.27±0.04 ng/L), ethyl acetate leaves fraction (0.22±0.06

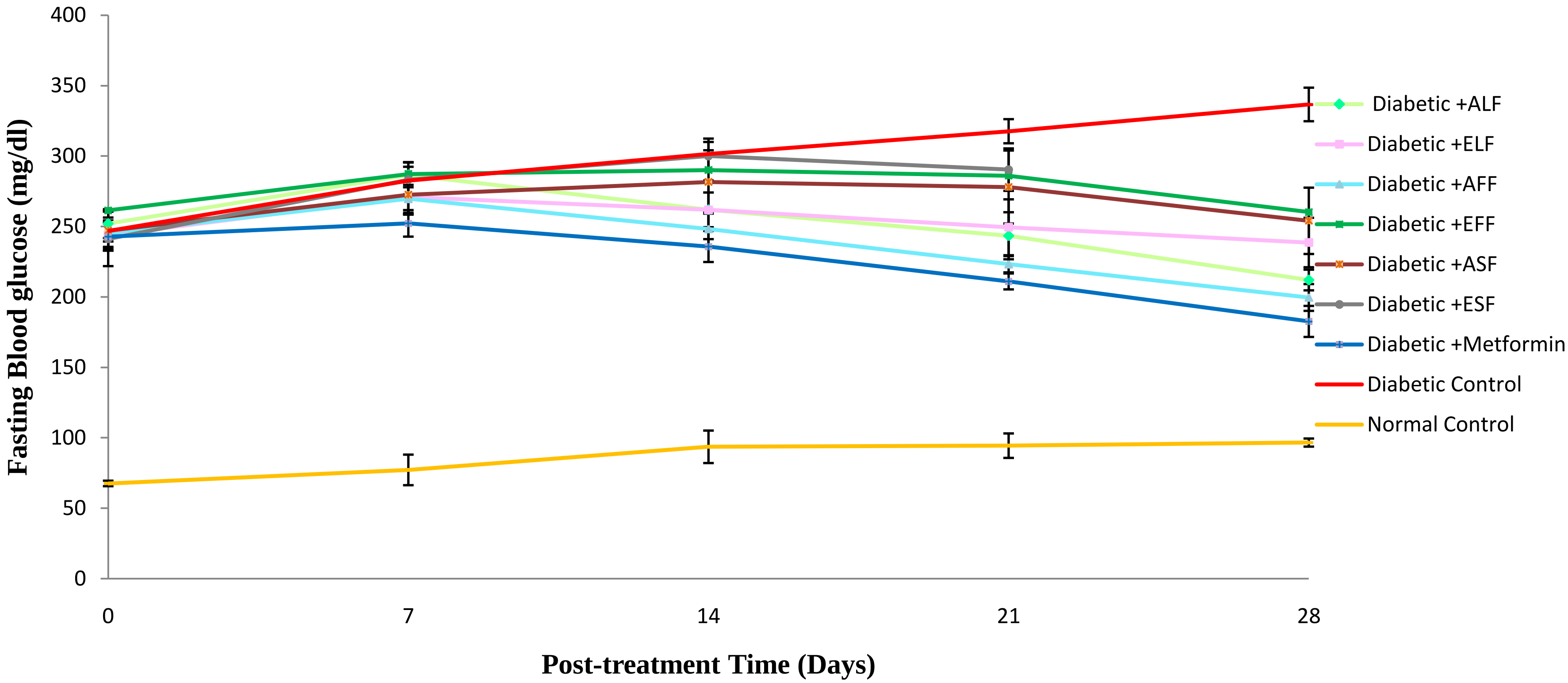


Figure 4.4 Antihyperglycemic Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-bark and Fruit-Mesocarp in Streptozotocin-induced Diabetic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF =

Ethyl AcetateFruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction

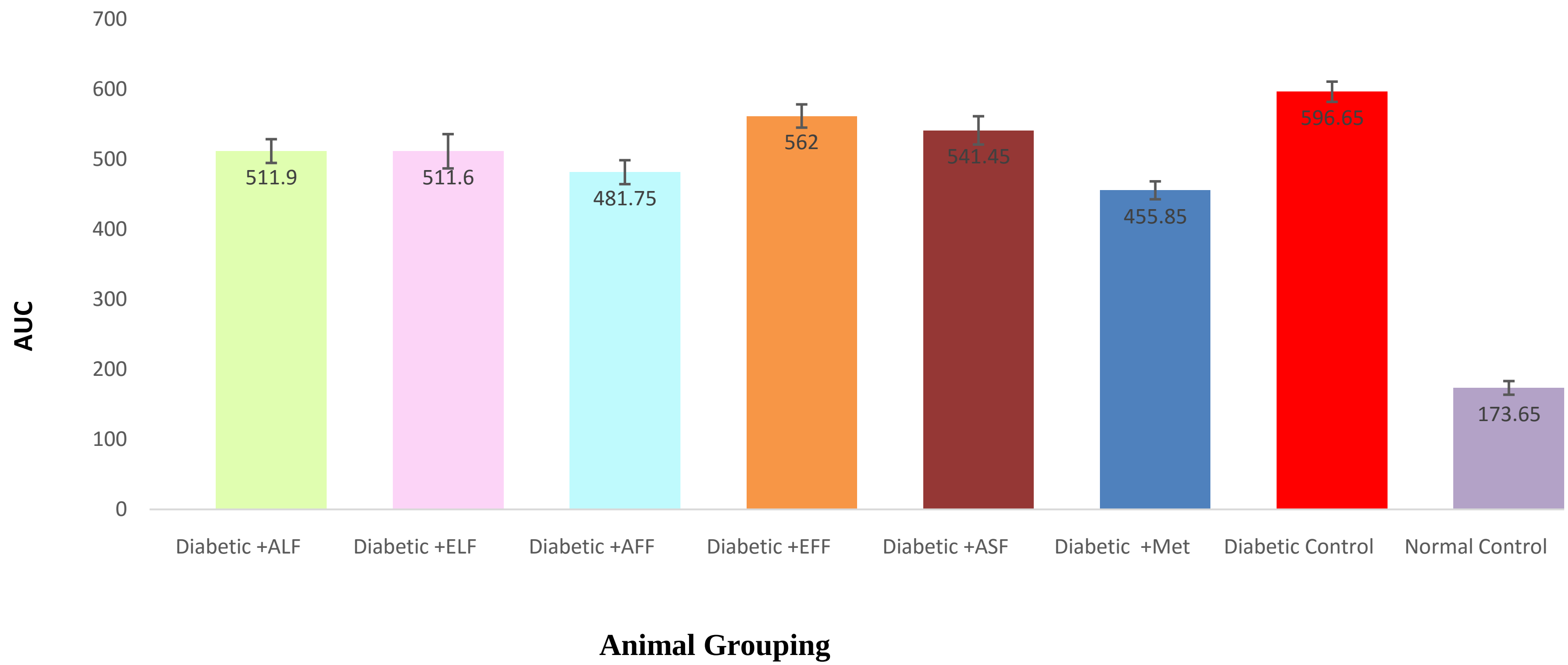


Figure 4.5Total Area Under Fasting Blood Glucose Curve of Streptozotocin-inducedDiabetic Rats Treated with Ethanol Extract-Fractions of*Balanites aegyptiaca* Leaves, Stem-bark and Fruit-mesocarp

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit fraction, EFF = Ethyl

Acetatefruit fraction, ASF = Aqueous stem-bark fraction, AUC = Area under the curve ng/L) and aqueous fruit-mesocarp fraction (0.32±0.10ng/L) were significantly different ($P<0.05$) compared to untreated diabetic rats (0.09±0.02 ng/L) and other extract-fractions used as presented in Figure 4.6.

4.7.3Effect of extract-fractions of *Balanites aegpytiaca*on lipids profile in streptozotocin-induced diabetic rats

STZ-induced diabetic rats showed significant increase in cholesterol, TG, VLDL but decrease in HDL level compared to normal and diabetic treated rats (Table 4.4). Administration of ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp to diabetic rats significantly reversed lipid profile levels. Serum cholesterol level in diabetic rats was 232.00±2.96 mg/dlwhich is significantly different($P<0.05$) compared to diabetic rats treated with metformin (180.62±3.19 mg/dl), aqueous leaves fraction (172.00±2.96 mg/dl) and aqueous fruit fraction (170.46±2.96 mg/dl). Similarly, elevated serum triglycerides levels were significantly ($P<0.05$) reduced in diabetic rats treated with aqueous leaves fraction (119.91±2.60 mg/dl) and aqueous fruit-mesocarp fraction (140.44±1.71 mg/dl) whereaselevated levels of HDL-cholesterol were recorded in same rats groups compared to diabetic untreated rats. Leaves and fruit-mesocarp (ethanol-aqueous fractions) of the plant are the most potent.

4.7.4Effect of extract-fractions of *Balanites aegpytiaca*on biochemical parameters in streptozotocin-induced diabetic rats

Result of hepatic glycogen content, hepatic ALT and AST enzymes, serum albumin, fructosamine and total protein of diabetic untreated and treated rats with ethanol extract-fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp are

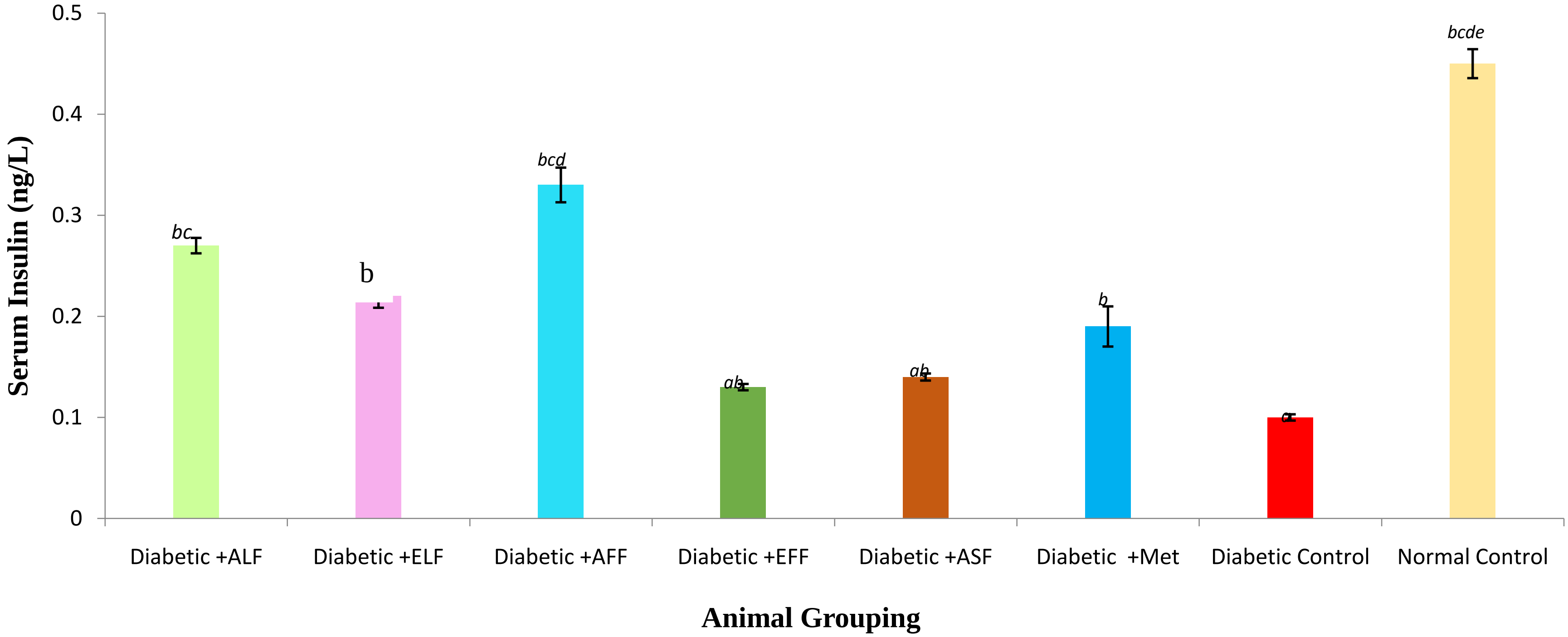


Figure 4.6 Effect of Oral Administration of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Insulin Level in Streptozotocin-induced Diabetic Rats

Bars with different letters are significantly different at $P<0.05$. ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction

Table 4.4 Antihyperlipidermic Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Streptozotocin-induced Diabetic Rats

	Animal Grouping							
	Diabetic + ALF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Cholesterol (mg/dL)	172.00± 2.96^{b}	224.61± 13.10^{bcde}	170.46± 2.96^{b}	217.85± 3.54^{bcde}	212.31± 2.34^{bcd}	232.00± 2.96^{bcdef}	180.62± 3.19^{bc}	76.31± 3.19^{a}
Triglyceride (mg/dL)	119.91± 2.60^{b}	185.39± 5.73^{bcd}	140.44± 1.71^{bc}	143.13± 3.46^{bc}	229.83± 3.32^{bcdef}	207.13± 6.05^{bcde}	99.91± 5.55^{a}	97.82± 2.90^{a}
HDL-Cholesterol (mg/dL)	50.00± 0.66^{bcd}	60.87± 1.59^{bcdef}	50.83± 0.95^{bcd}	55.49± 7.90^{bcde}	39.47± 2.43^{b}	27.22± 5.07^{a}	66.35± 5.53^{bcdef}	47.78± 3.01^{bc}
LDL-Cholesterol (mg/dL)	138.02± 3.03^{b}	175.37± 12.98^{bcde}	132.21± 3.20^{b}	178.12± 3.71^{bcdef}	158.45± 2.59^{bcd}	185.13± 4.78^{bcdef}	147.36± 4.57^{bcd}	47.19± 3.56^{a}
VLDL (mg/dL)	23.98± 0.52^{b}	37.07± 1.15^{bcd}	28.09± 0.34^{bc}	28.63± 0.69^{bc}	45.97± 0.66^{bcdef}	41.43± 1.21^{bcde}	19.98± 1.11^{a}	19.57± 0.58^{a}

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction presented in Table 4.5. There was a significant reduction (*P<0.05*) in glycogen content of diabetic untreatd rats (10.69±0.32 mg/g liver) in comparison tothe diabetic treated rats. Significant elevation (*P<0.05*) in glycogen content was observed indiabetic rats that received aqueous leaves fraction (18.31±0.44 mg/g liver) and aqueous fruit-mesocarp fraction (20.62±0.44 mg/g liver). Similar elevation was noticed in glycogen concentration of the metformin-treated diabetic rats (17.77±0.32 mg/g liver).

The results of our study showed a reduction in fructosamine level by extract-fractions of *Balanites aegyptiaca* parts which is significant (*P<0.05*) compared to metformin treated diabetic rats group. There was significant elevation (*P<0.05*) in the activities of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the rats groups that received plant extract-fractions compared to the normal control. In contrast, there was significant reduction (*P<0.05*) in enzymes activities in the groups treated with metformin compared to normal and diabetic control rats groups. There was significant (*P<0.05*) decrease in the concentration of albumin (ALB) of untreated diabetic rats (2.21±0.16 g/dl) compared to all the diabetic treated groups.

4.7.5Effect of extract-fractions of *Balanites aegpytiaca*on packed cell volume (PCV) in streptozotocin-induced diabetic rats

The PCV of STZ-induced diabetic rats following administration of ethanol extract-fractions of *Balanites aegyptiaca*parts are presented in Figure 4.7.

Fluctuation of PCV in diabetic rats group treated with the ethanol extract-fractions

Table 4.5 Effect of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Fruit-Mesocarp, and Stem-Bark onBiochemical Parameters in Streptozotocin-induced Diabetic Rats

	Animal Grouping							
	Diabetic + ALF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Glycogen (mg/g liver)	18.31± 0.44^{bcd}	18.15± 1.52^{bcd}	20.62± 0.44^{bcde}	14.54± 0.32^{b}	13.77± 0.32^{b}	10.69± 0.32^{a}	17.77± 0.32^{bcd}	15.85± 0.32^{bc}
Fructosamine (mmol/L) $x10^{-1}$	0.83± 0.19^{b}	0.67± 0.12^{ab}	0.67± 0.01^{ab}	0.56± 0.12^{ab}	0.53± 0.01^{ab}	2.91± 0.70^{bc}	0.48± 0.01^{ab}	0.30± 0.16^{a}
Albumin (g/dL)	4.00± 0.01^{bcde}	2.44± 0.01^{b}	3.94± 0.14^{bcde}	4.24± 0.01^{bcdef}	2.57± 0.01^{bc}	2.21± 0.16^{a}	3.59± 0.00^{bcd}	4.28± 0.01^{bcdef}
Total Protein (mg/g liver)	21.58± 0.06^{bcd}	20.85± 0.17^{bc}	19.63± 0.02^{b}	21.06± 0.06^{bcd}	19.76± 1.93^{bc}	17.35± 1.96^{a}	25.01± 0.06^{bcde}	26.95± 0.04^{bcdef}
ALT (U/L)	9.04± 0.16^{bcdef}	5.20± 0.14^{bcd}	6.08± 0.18^{bcde}	5.96± 0.78^{bcde}	4.44± 0.33^{bc}	4.28± 0.23^{bc}	3.24± 0.82^{a}	3.48± 0.11^{a}
AST (U/L)	13.50± 1.66^{bcdef}	12.80± 0.57^{bcd}	11.50± 0.35^{b}	9.60± 1.67^{a}	22.80± 2.41^{bcdefg}	13.30± 0.27^{bcde}	12.90± 0.65^{bcd}	11.70± 0.27^{bc}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction, ALT = Alanine Transaminase, AST = Aspartate Transaminase was observed. However, thelevels were still within the normal range.

4.7.6Effect of extract-fractions of *Balanites aegpytiaca*on body weight of streptozotocin-induced diabetic rats

Change in body weight of STZ-induced diabetic rats following treatment with ethyl acetate and aqueous fractions of *Balanites aegyptiaca* fruit-mesocarp is presented in Figure 4.8. Body weight of the diabetic treated and untreated rats in general decreased after 7 days which continued throughout the experimental period in the untreated diabetic rats. In the diabetic treated rats, the study recorded a gain in body weight by the rats as the treatment advances. However, diabetic rats that received extract-fractions of the plant stem-bark hadlow body weight gain comparedto other treated rats groups. But, in generalthe study recorded a gain in body weight by diabetic treated compared to untreated rats.

4.7.7Effect of extract-fractions of *Balanites aegpytiaca*on water and feed intake of streptozotocin-induced diabetic rats

The feed and water intake of STZ-induced diabetic treated, diabetic untreated, and non-diabetic rats groups are presented in Table 4.6. No significant (P>0.05) change was observed in feed or water intake among the STZ-induced diabetic rats that received plant extract-fractions and metformin, but significant ($P<0.05$) change was recorded compared to diabetic and normal control rats groups.

4.7.8Effect of extract-fractions of *Balanites aegpytiaca* on glycolytic enzymes in streptozotocin-induced diabetic rats

Activities of glycolytic enzymes namely; glucokinase (GK), phosphofructo-

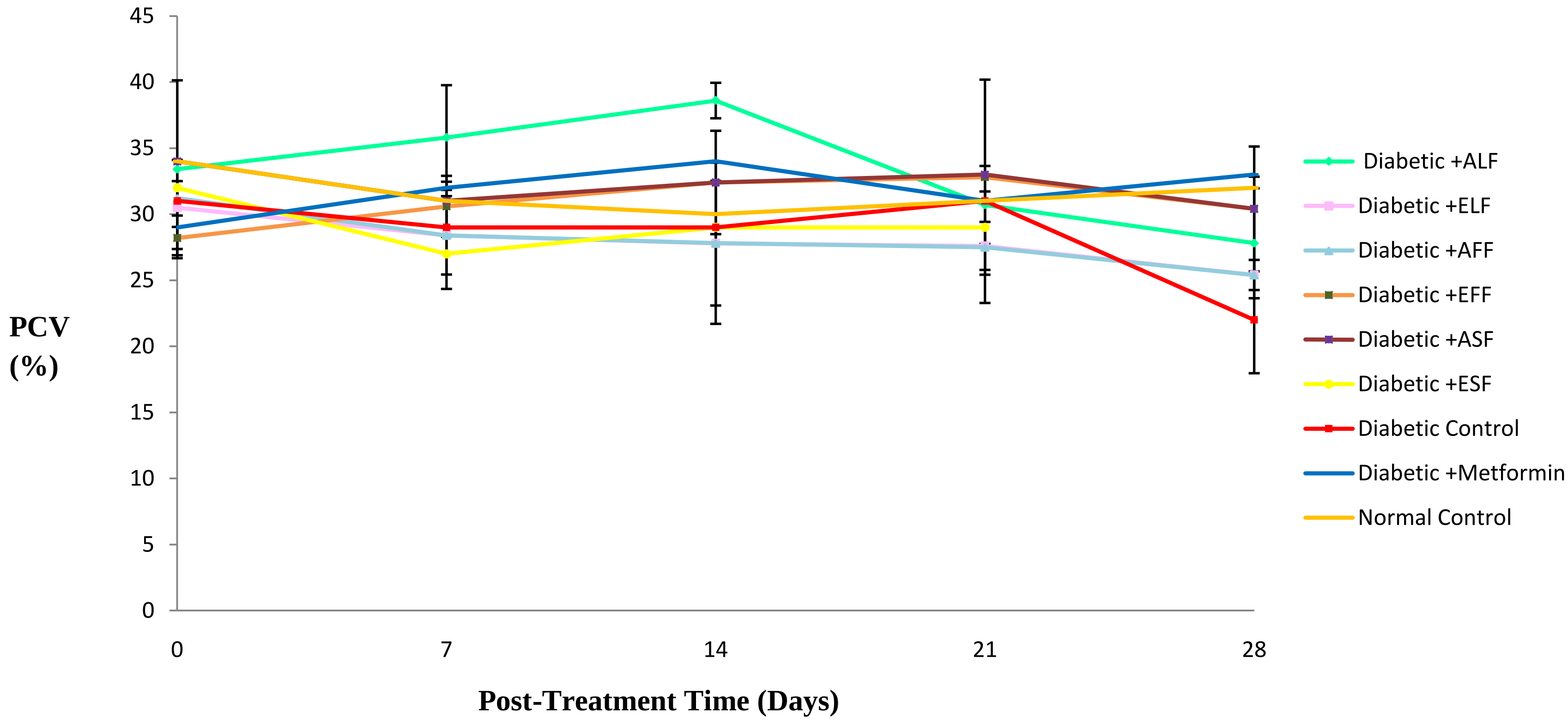


Figure 4.7Effect of Oral Administration of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on PCV in Streptozotocin-induced Diabetic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetateFruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction

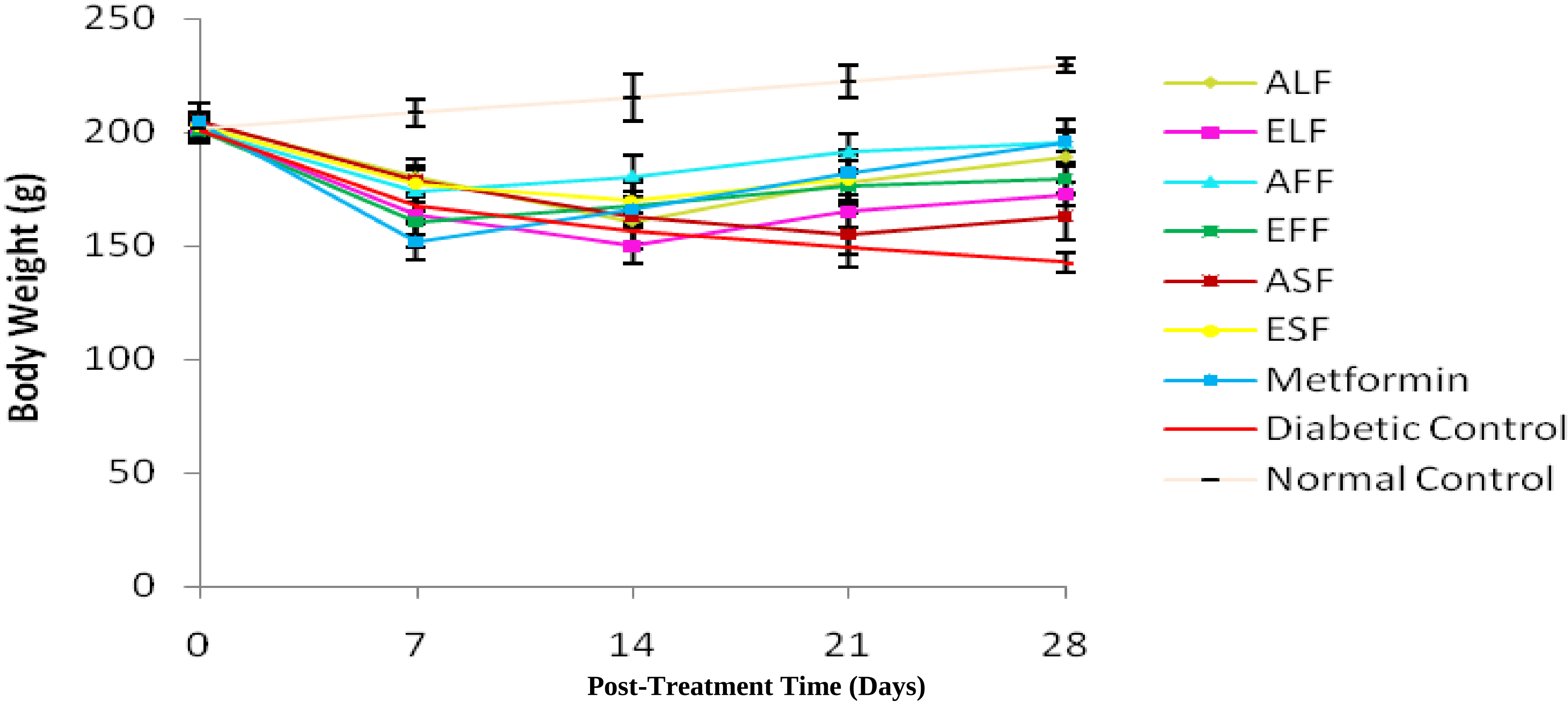


Figure 4.8Effect of Oral Administration of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Stem-Bark and Fruit-Mesocarp on Body Weight of Streptozotocin-Induced Diabetic Rats

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetateFruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction

Table 4.6 Effect of Oral Administration of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves, Fruit- Mesocarp and Stem-Bark on Feed and Water Intake of Streptozotocin-induced Diabetic Rats

	Animal Grouping							
	Diabetic + ALF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Feed (g/day/rat)	29.59± 4.23^{b}	26.67± 8.41^{b}	27.67± 2.54^{b}	27.99± 2.97^{b}	27.51± 1.72^{b}	32.37± 3.63^{bc}	25.37± 3.50^{b}	23.27± 6.51^{a}
Water (ml/day/rat)	23.86± 5.84^{b}	26.63± 7.98^{b}	26.35± 7.40^{b}	25.59± 6.62^{b}	24.98± 6.64^{b}	29.04± 9.15^{bc}	21.47± 4.91^{b}	17.67± 2.19^{a}

Values are Mean ± SD of 28determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate Fruit-mesocarpfraction, ASF = Aqueous Stem-bark fraction kinase(PFK), and pyruvate kinase (PK) determined in liver tissues of STZ-induced diabetic rats treated with ethanol extract-fractions of *Balanitesaegyptiaca* parts and metformin for 28 days are presented in Table 4.7. Changes were observed in the activities of these enzymes.Activities of these key enzymes assayed in liver tissues of diabetic control rats were suppressed. However, the enzymes activities of diabetic rats orally administered various parts of the plant extract-fractions were reversed significantly (P<0.05) by varying degrees as compared to the diabetic untreated rats.

Balanites aegyptiaca fruit-mesocarp (aqueous-fraction)was more effective on glucokinase (from 2.22±0.02 to 3.58±0.05 U/min/mg protein) compared to the other extract-fractions. Whereas,the leaves extract (aqueous leaves fraction) was effective on phosphofructokinase activity (from 2.06±0.07 to 2.52±0.03 U/min/mg protein) compared to the other extract-fractions.

4.7.9Effect of extract-fractions of *Balanites aegpytiaca*on glycogen metabolic enzymes in streptozotocin-induced diabetic rats

Activities of glycogen metabolic enzymes; glycogen synthase (GS) and phosphorylase in liver of diabetic untreated,non-diabetic and diabetic rats treated with ethylacetate and aqueous fractions of *Balanites aegpytiaca*parts and metformin are presented in Table 4.8. A significant (*P<0.05*) increased in glycogen phosporylase activity was recorded in the diabetic control rats group (3.82±0.21 U/min/mg protein) compared with metformin treated rats group (2.04±0.01 U/min/mg protein) and the plant ethanol extract-fractions as recorded.

Table 4.7 Effect of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Fruit-Mesocarp and Stem-Bark onGlycolytic Enzymes Activities in Liver of Streptozotocin-inducedDiabetic Rats

	Animal Grouping							
	Diabetic + ALF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Glucokinase (U/min/mg Protein)	2.75± 0.01^{b}	2.23± 0.00^{a}	3.58± 0.05^{bcde}	3.10± 0.02^{bcd}	2.98± 2.23^{bcd}	2.22± 0.02^{a}	2.72± 0.02^{b}	3.53± 0.01^{bcde}
Phosphofructokinase (U/min/mg Protein)	2.52± 0.03^{bc}	2.23± 0.01^{b}	2.30± 0.57^{b}	1.25± 0.03^{a}	2.73± 0.11^{bc}	2.06± 0.07^{ab}	3.34± 0.01^{bcd}	4.43± 0.08^{bcde}
Pyruvate Kinase (U/min/mg Protein) $x10^{-1}$	0.22± 0.03^{bcd}	0.14± 0.02^{bc}	0.30± 0.03^{bcde}	0.09± 0.03^{b}	0.08± 0.01^{b}	0.04± 0.01^{a}	0.15± 0.02^{bc}	0.11± 0.01^{b}
LDH (U/min/mg Protein)$x10^{-2}$	11.67± 0.30^{bc}	10.87± 0.02^{bc}	6.07± 2.05^{ab}	7.13± 2.69^{b}	6.22± 0.58^{ab}	4.36± 1.58^{a}	6.12± 2.19^{ab}	7.10± 1.60^{b}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethylacetate fruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction, LDH = Lactate Dehydrogenase

In line with the results of the glycogen phosphorylase activity;*Balanites aeygptiaca* leaves (aqueous and ethyl acetatefractions)were the most effective on glycogen phosphorylase suppression (from 3.82±0.21 to 2.76±0.02 U/min/mg protein). Significant effect bythe aqueous leaves fraction(ALF) (14.45±0.16 x10^{-2}U/min/mg protein) on glycogen synthase (GS); was recorded compared to other ethanol extract-fractions used and diabetic control (9.41±0.34 x10^{-2}U/min/mg protein) (Table 4.8).

4.7.10Effect of extract-fractions of *Balanites aegpytiaca*on gluconeogenic enzymes in streptozotocin-induced diabetic rats

Changes in the activities of hepatic gluconeogenic enzymes namely glucose-6-phosphatase (GPase), fructose-1,6-bisphosphatase (FBPase), and phosphoenol pyruvate carboxyl kinase (PEPCK) in diabetic untreated, diabetic treated and non-diabetic rats are shown in Table 4.9. The diabetic untreated rats showed increase in their gluconeogenic enzymes activity. However, these were significantly ($P < 0.05$) suppressed in the diabetic treated animals. Aqueous leaves fraction (ALF) was the most effective as shown; glucose-6-phosphatase (from 1.44±0.05 to0.14±0.01 U/min/µmole P_i liberated), fructose-1,6-bisphosphatase (from 2.19±0.25 to 1.32±0.06U/min/µmole P_i liberated), phosphoenol pyruvate carboxylkinase (0.17±0.01 U/min/mg protein) compared to other extract-fractions used.

4.7.11Effect of extract-fractions of *Balanites aegpytiaca*on pentose phosphate metabolic enzyme in streptozotocin-induced diabetic rats

Activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined in the

Table 4.8 Effectof Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Fruit-Mesocarp and Stem-Bark onGlycogen Metabolic Enzymes Activities inLiver of Streptozotocin-induced Diabetic Rats

	Animal Grouping							
	Diabetic + ALF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Glycogen Phosphorylase (U/min/mg Protein)	2.76± 0.02^{b}	2.75± 0.01^{b}	3.75± 0.00^{bcde}	3.46± 0.01^{bcd}	3.14± 0.07^{bc}	3.82± 0.21^{bcde}	2.04± 0.01^{a}	2.07± 0.01^{a}
Glycogen Synthase (U/min/mg Protein) x10^{-2}	14.45± 0.16^{bcde}	12.00± 0.22^{bc}	12.24± 0.22^{bc}	11.75± 0.11^{b}	12.48± 0.11^{bcd}	9.41± 0.34^{a}	15.51± 0.42^{bcdef}	29.25± 0.88^{bcdefg}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction, ASF = Aqueous Stem-bark fractio

Table 4.9 Effect of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Fruit-Mesocarp, and Stem-Bark on GluconeogenicEnzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

	Animal Groups							
	Diabetic + A LF	**Diabetic + ELF**	**Diabetic + AFF**	**Diabetic + EFF**	**Diabetic + ASF**	**Diabetic Control**	**Diabetic + Metformin**	**Normal Control**
Glucose-6-Phosphatase (U/min/µmole P_i liberated)	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.17 ± 0.00^{a}	0.70 ± 0.01^{b}	0.26 ± 0.03^{ab}	1.44 ± 0.05^{bc}	0.12 ± 0.02^{a}	0.07 ± 0.01^{a}
Fructose-1,6-Bis-Phosphatase (U/min/µmole P_i liberated)	1.32 ± 0.06^{bc}	1.43 ± 0.17^{bcd}	1.41 ± 0.06^{bcd}	1.20 ± 0.03^{b}	1.42 ± 0.09^{bcd}	2.19 ± 0.25^{bcde}	1.02 ± 0.02^{a}	1.40 ± 0.07^{bcd}
Phosphoenol-pyruvate Carboxyl kinase (U/min/mg Protein)	0.17 ± 0.01^{ab}	0.21 ± 0.01^{b}	0.38 ± 0.04^{bc}	0.46 ± 0.01^{bcd}	0.20 ± 0.01^{b}	0.81 ± 0.15^{bcde}	0.11 ± 0.04^{a}	0.09 ± 0.01^{a}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl Acetate Fruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction liver tissues of diabetic treated rats in order to assess the impact of ethyl acetate and aqueous fractions of *Balanites aegyptiaca*parts (Figure 4.9). Treatmentwith metformin and ethanolextract-fractions of *Balanites aegyptiaca* leaves, fruit-mesocarp and stem-barkenhanced G6PDH activity by varying degrees. *Balanites aegyptiaca*fruit-mesocarp (aqueous fraction, AFF) was more effective in enhancing G6PDH activity (from 1.45±0.02 to 2.10±0.02 U/min/mg protein) compared with values obtained from diabetic rats groups that received the other extract-fractions.

4.8 Yields, Phenolics and Flavonoids Content of SubFractions Derived FromEthanol-Aqueous Fraction of *Balanites aegyptiaca*Leaves

Yields, total phenolics and flavonoids content of sub-fractions obtained from the column fractionation of ethanol-aqueous fraction of *Balanites aegyptiaca* leaves is shown in Table 4.10. The leavessub-fractions varied in yield and the amount of both phenolics and flavonoids. Methanol sub-fraction (L4) has the highest percent yield (13.2 %) out of the 150 g of ALF fractionated. Its total phenolics was $6.32 \pm 0.94 \times 10^{-2}$out of the $66.08 \pm 6.07 \times 10^{-2}$mg g^{-1}GAE in ALF whereas diethyl ether /chloroform leavessubfraction (L2) recorded the highest amount of total flavonoids ($0.31 \pm 0.06 \times 10^{-2}$out of the $3.45 \pm 0.04 \times 10^{-2}$mg g^{-1}QE in ALF).

4.9 TLC Analysis of Sub-Fractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaves

TLC analysis of sub-fractions obtained fromthe column fractionation of ethanol-aqueous fraction of *Balanites aegyptiaca*leaves using solvent system comprises of butanol, acetic acid and water (6:3:1). TLC chromatograms is presented in Plate I. Retention factor (Rf) values calculated are presented in Table 4.11.

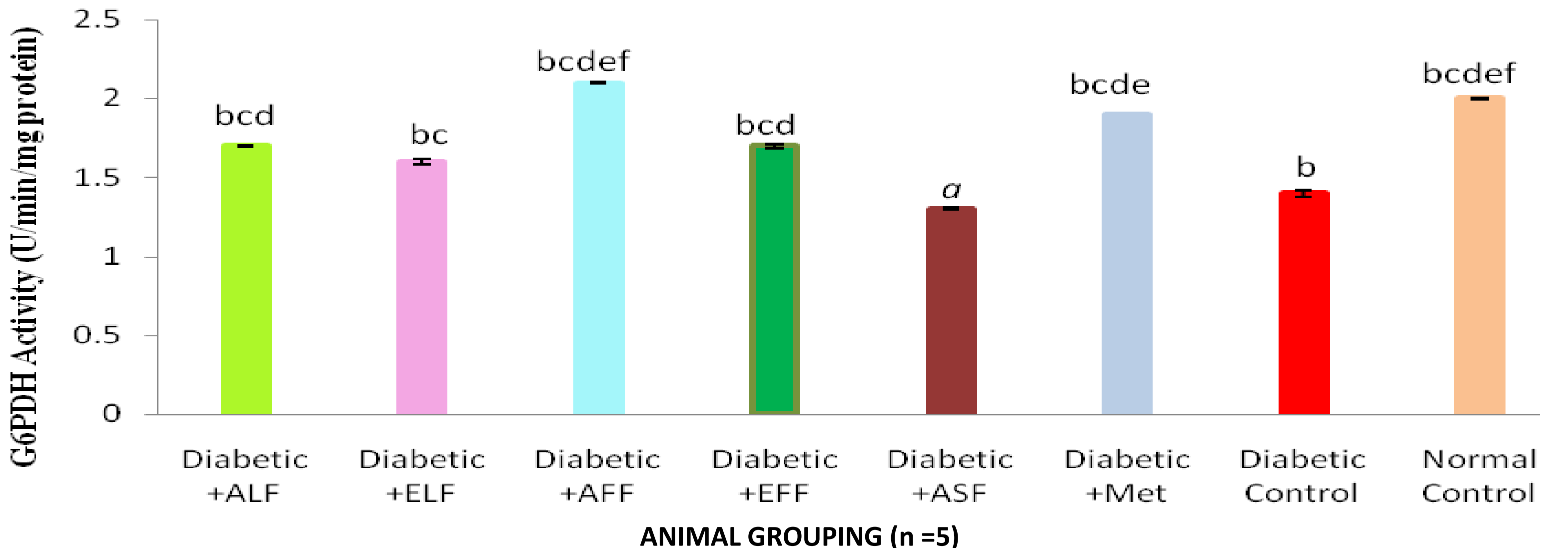


Figure 4.9 Effect of Ethanol Extract-Fractionsof *Balanites aegyptiaca* Leaves, Fruit-Mesocarp and Stem-bark on Glucose 6-phosphadehydrogenase Activity in STZ-induced Diabetic Rats

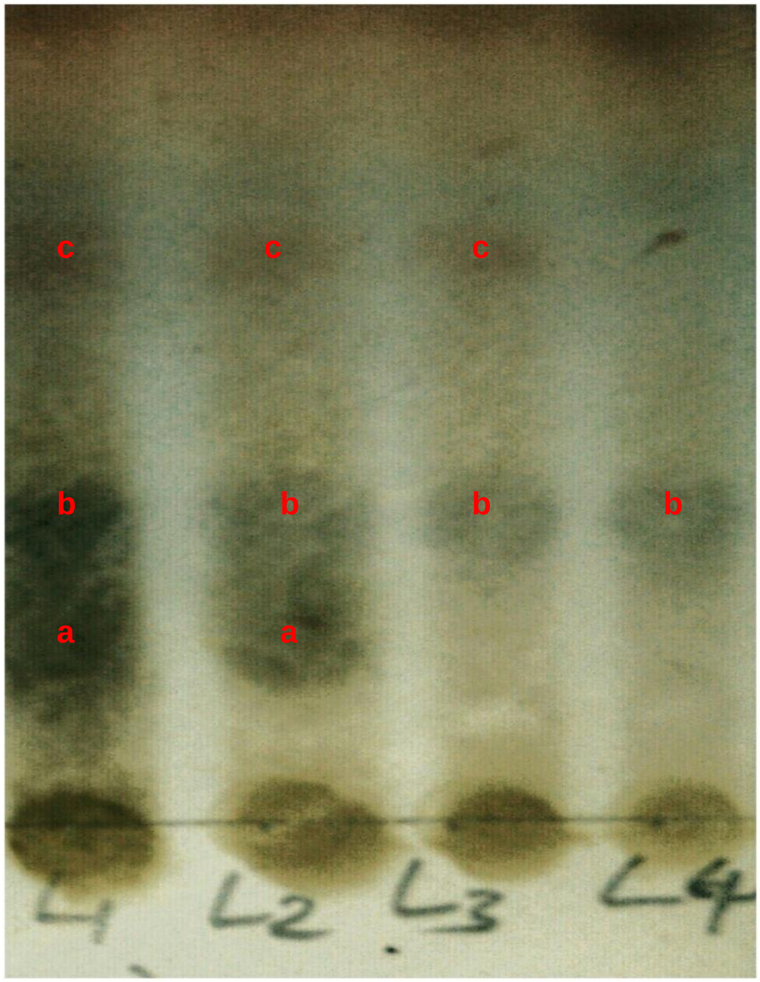
Bars with different letters are significantly different at $P<0.05$. ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate Fruit-mesocarp fraction, ASF = Aqueous Stem-bark fraction, G6PDH= Glucose-6-phosphate Dehydrogenase

Table 4.10 Yield and Total Phenolics/Flavonoids Content of Sub-Fractions Derived from Ethanol-Aqueous Fractionof *Balanites aegyptiaca* Leaves

	Subfractions			
	L1	L2	L3	L4
Yield (g/150g)	15.0 (10.0 %)	13.2(8.8 %)	17.7(11.8 %)	19.8(13.2 %)
Phenolics (mg/GAE/g)x10^{-2}	5.54 ± 0.29^{bc}	4.76 ± 0.45^{b}	3.21 ± 0.29^{a}	6.32 ± 0.94^{bc}
Flavonoids (mg /QE/g) x10^{-2}	0.10 ± 0.00^{a}	0.31 ± 0.06^{bc}	0.17 ± 0.01^{b}	0.23 ± 0.00^{b}

Values are Mean ± SD of triplicate determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction, **L3** =chloroform leaves subfraction, **L4** = Methanol leaves subfraction



PlateIII.TLC Chromatogram of Subfractions Following Column Fractionation of Ethanol-Aqueous Fractionof *Balanites aegyptiaca* Leaves

Letter a, b and c designated spots of various component in the extract-subfractions. **L1** = Diethyl ether leavessubfraction, **L2** = Diethyl ether/chloroform leavessubfraction, **L3** = Chloroform leavessubfraction, **L4** = Methanol leavessubfraction

Table 4.11 RF Values of TLC for Subfractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaves

	Subfractions			
Components	L1	L2	L3	L4
A	0.12 ±0.02	0.12 ±0.02	-----	-----
B	0.29 ±0.06	0.29 ±0.06	0.29 ±0.06	0.29 ±0.06
C	0.38 ±0.06	0.38 ±0.04	0.38 ±0.04	0.38 ±0.04

Values are Mean ± SD of triplicate determinations

L1 = Diethyl ether leavessubfraction, **L2** = Diethyl ether/chloroform leavessubfraction, **L3** = Chloroform leavessubfraction, **L4** = Methanol leavessubfraction

4.10 Effect of Subfractions in Streptozottocin-Induced Diabetic Rats

4.10.1 Anti-hyperglycemic effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves in diabetic rats

The anti-hyperglycemic effect of various sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leavesin streptozotocin-induced diabetic rats is presented in Figure 4.10. The study recorded high levels of fasting blood glucose in the diabetic treated rats at the early peroid of administration of the subfractions but as the treatment advances the fasting blood glucose levels reduced significantly. Diabetic rats group that received methanolleavessubfraction (L4) and the Metformin treated group had low value of area under fasting blood glucose curve but high percent of FBG reduction 19.18 % and 22. 93 % by metformin compare to diabetic rats groupsthat were treated with the other subfractions (figure 4.11).

4.10.2 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on biochemical parameters in diabetic rats

There was significant ($P<0.05$) change in cholesterol, TG, HDL, LDL levels in the diabetic rats groups treated with diethyl ether, dithyl ether/chloroform, chloroform and methanol subfractions (Table 4.12). The sub-fraction that was more effective was the methanol(L4) leavessubfractions which lowered cholesterol from 205.17±6.09 to 110.00±12.75 mg/dL, TG from 271.96±9.06 to 118.31±11.9 mg/dL compare to Metformin treated group where cholesterol was lowered to 106.21 15.35 mg/dL and TG (103.55±10.09 mg/dL.Serum insulin levels among the diabetic treated rats groups were not significantly different ($P>0.05$) but were significantly different ($P<0.05$) compare to diabetic untreated and normal control rats.

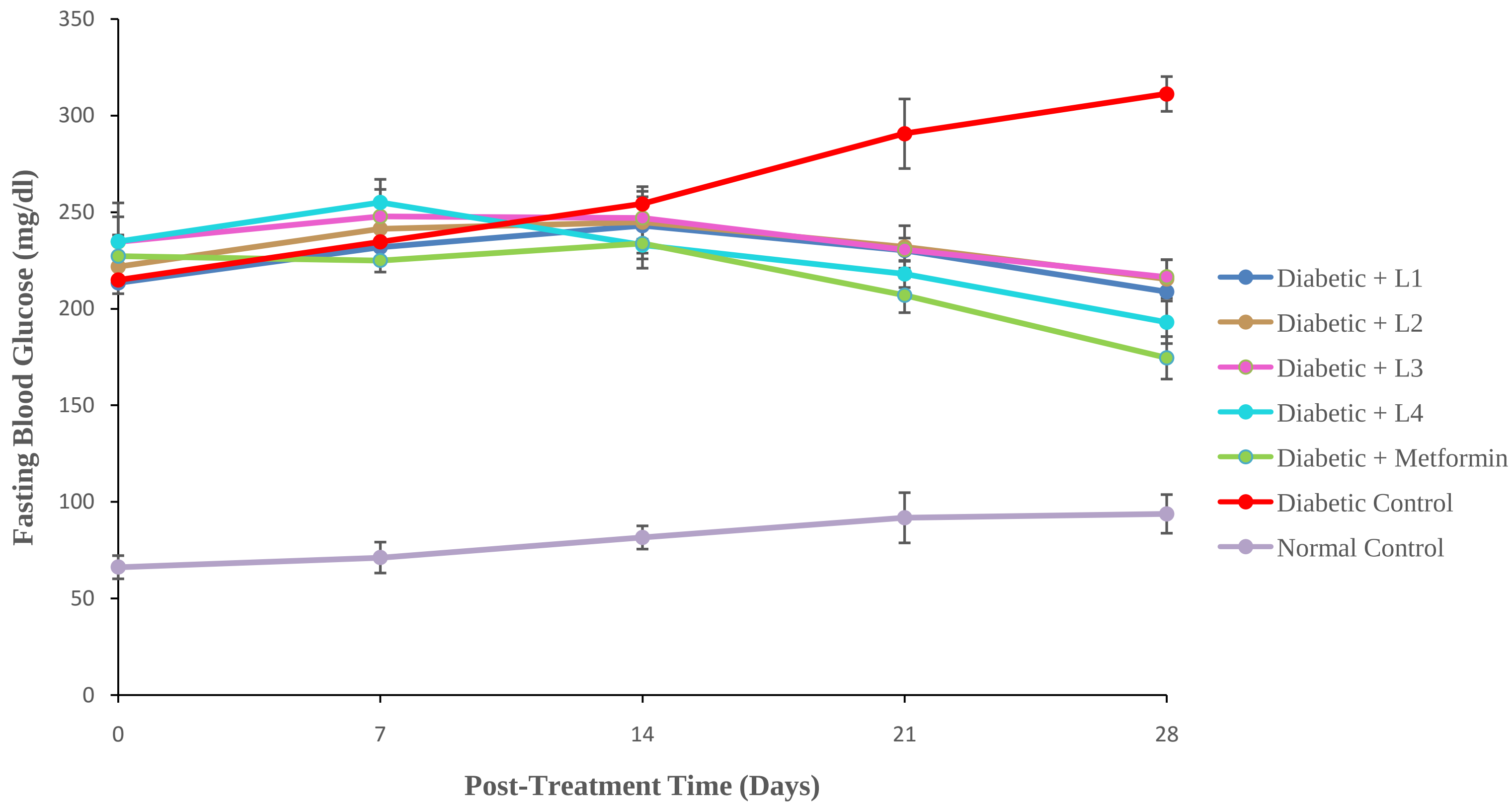


Figure 4.10 Anti-hyperglycemic Effect of Sub-Fractions Derived fromEthanol-Aqueous Fraction of *Balanites aegyptiaca* Leavesin Streptozotocin-induced Diabetic Rats

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction,**L3**=chloroform leaves subfraction, **L4** = Methanol leaves subfraction

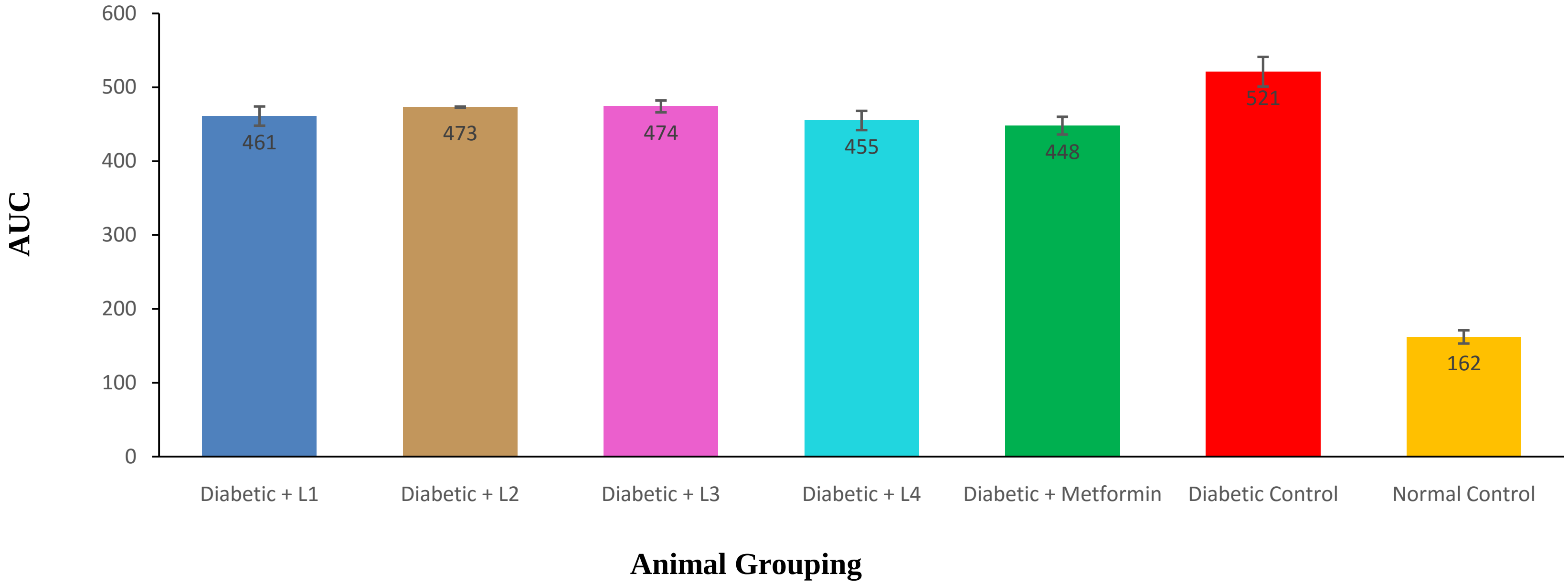


Figure 4.11 Total Area Under Fasting Blood Glucose Curve of Streptozotocin-induced Diabetic Rats Treated with Subfractions Derived from Ethanol Aqueous-Fractions of *Balanites aegyptiaca* Leaves

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction, **L3** =chloroform leaves subfraction, **L4** = Methanol leaves subfraction

Table 4.12 Effect of Sub-Fractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaves on Biochemical Parametersin Streptozotocin-induced Diabetic Rats

	Animal Grouping						
	Diabetic + L1	**Diabetic + L2**	**Diabetic + L3**	**Diabetic + L4**	**Diabetic + Metformin**	**Diabetic Control**	**Normal Control**
Cholesterol (mg/dL)	190.69 ± 11.48^{bcd}	197.24 ± 5.26^{bcd}	162.07 ± 14.52^{bc}	110.00 ± 12.75^{b}	106.21 ± 15.35^{b}	205.17 ± 6.09^{bcd}	81.38 ± 12.03^{a}
Triglyceride (mg/dL)	197.20 ± 8.39^{bcd}	199.81 ± 25.09^{bcd}	142.99 ± 42.37^{bc}	118.31 ± 11.9^{b}	103.55 ± 10.09^{ab}	271.96 ± 9.06^{bcde}	80.75 ± 11.60^{a}
HDL-C(mg/dL)	54.78 ± 3.44^{b}	50.54 ± 1.33^{b}	55.96 ± 1.58^{b}	68.97 ± 4.30^{bc}	76.36 ± 12.03^{bcd}	41.58 ± 7.65^{a}	84.63 ± 7.43^{bcd}
LDL-C(mg/dL)	140.30 ± 10.12^{bcd}	147.17 ± 7.99^{bcd}	122.28 ± 8.90^{bc}	72.54 ± 14.82^{b}	70.23 ± 12.30^{b}	142.46 ± 4.59^{bcd}	48.30 ± 13.79^{a}
VLDL (mg/dL)	39.44 ± 1.45^{bcd}	39.96 ± 5.02^{bcd}	28.60 ± 8.47^{bc}	23.66 ± 2.39^{b}	20.71 ± 2.02^{ab}	54.39 ± 1.81^{bcde}	16.15 ± 2.32^{a}
Serum Insulin(pg/mL)	75.97 ± 13.98^{b}	75.81 ± 6.04^{b}	82.26 ± 11.69^{b}	83.15 ± 4.85^{b}	84.35 ± 13.41^{c}	66.29 ± 6.70^{a}	70.66 ± 19.19^{b}
Glycogen (mg/g liver)	13.55 ± 1.45^{b}	12.20 ± 0.76^{ab}	14.75 ± 1.86^{bc}	19.90 ± 2.07^{bcd}	22.00 ± 1.05^{bcde}	11.20 ± 1.24^{a}	23.95 ± 1.55^{bcdef}
Fructosamine(mmol/L)	0.37 ± 0.02^{bcd}	0.40 ± 0.02^{bcd}	0.33 ± 0.01^{bc}	0.26 ± 0.02^{b}	0.21 ± 0.05^{ab}	0.50 ± 0.04^{bcde}	0.20 ± 0.04^{a}
Albumin(g/dL)	2.76 ± 0.27^{b}	2.90 ± 0.47^{b}	3.30 ± 0.25^{bc}	3.68 ± 0.06^{bcd}	4.21 ± 0.23^{bcde}	2.14 ± 0.21^{a}	5.12 ± 0.28^{bcdef}
Total Protein (mg/g Liver)	16.45 ± 0.57^{a}	15.68 ± 0.34^{a}	16.71 ± 0.53^{a}	18.55 ± 1.05^{b}	21.03 ± 1.71^{bc}	16.29 ± 0.96^{a}	23.55 ± 0.57^{bcd}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different ($P<0.05$)
L1 = Diethyl ether leavessubfraction, L2 = Diethyl ether/chloroform leavessubfraction, L3 =chloroform leavessubfraction, L4 = Methanol leavessubfraction, HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol

4.10.3 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on body weight of streptozotocin-induced diabetic rats

The body weight of STZ-induced diabetic untreated and treated rats with sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves are presented in Figure 4.12. Body weight of diabetic untreated rats decreased graduallyand continued throughout the experimental period while that of the treated diabetic rats groups decreases at the initial of the experiment but later there was gain in body weight by all diabetic rats treated groups.

4.10.4 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on water and feed intake of streptozotocin-induced diabetic rats

Feed and water intake of STZ-induced diabetic treated, diabetic untreated, and non-diabetic rats groups are presented in Table 4.13. There were significant (P<0.05) difference observed in the feed and water intake of STZ-induced diabetic rats treated with sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves and diabetic untreated and normal control rats. Diabetic untreated has highest feed and water intake while normal rats recorded the least intake.

4.10.5 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on glycolytic enzymes in diabetic rats

Effects of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaveson key glycolytic enzymes: glucokinase (GK), phosphofructokinase (PFK), and pyruvate kinase (PK) activities in liver tissues of streptozotocin-induced diabetic rats is presented in Table 4.14. Significant changes ($P<0.05$) were observed in the activities of these enzymes. Diabetic rats groups that received particularly the methanol (L4) leaves subfraction had their glucokinase, phosphofructokinase and

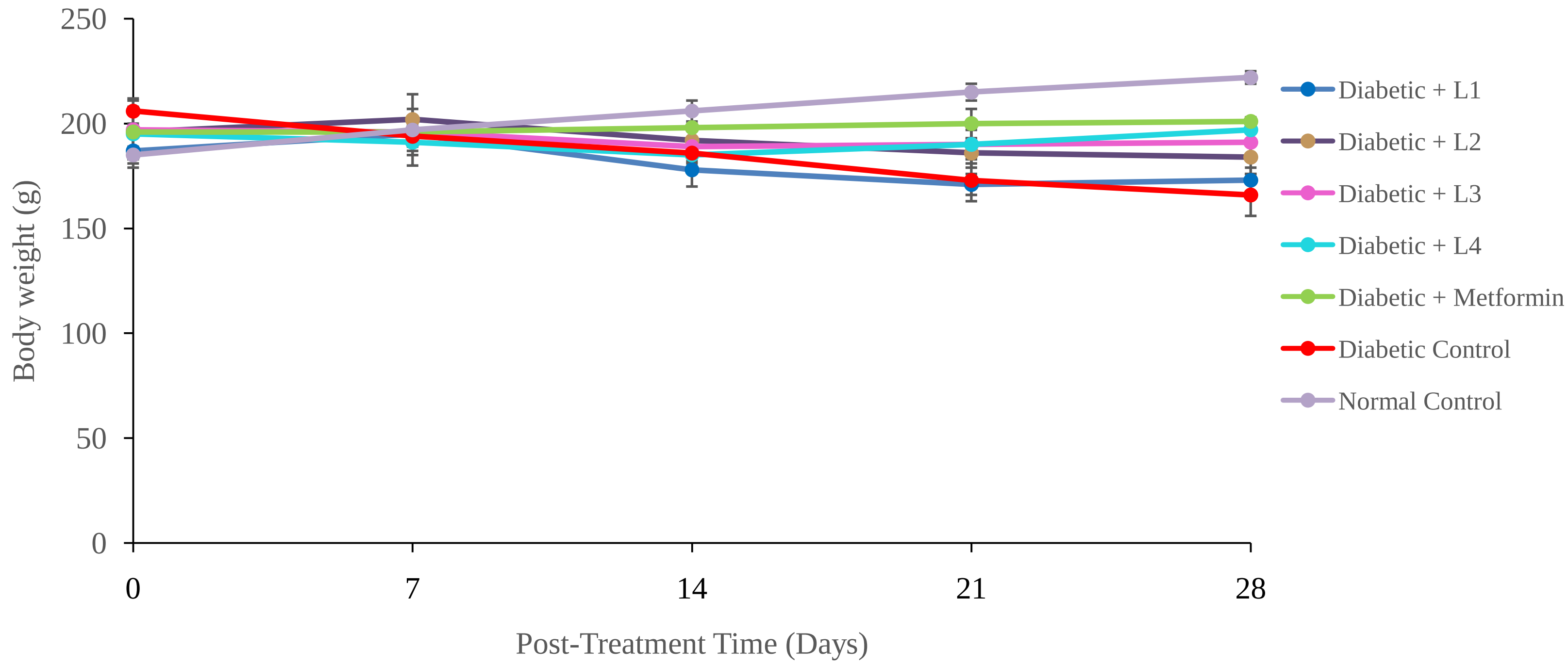


Figure 4.12Effect of Oral Administration of Sub-Fractions Derived From Ethanol-Aqueous Fraction of *Balanites aegyptiaca*Leaves on Body Weight of Streptozotocin-Induced Diabetic Rats

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction, **L3** =chloroform leaves subfraction, **L4** = Methanol leaves subfraction

Table 4.13 Effect of Sub-Fractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaves on Water and Feed Intake in Streptozotocin-induced Diabetic Rats

	Animal Grouping						
	Diabetic + L1	**Diabetic + L2**	**Diabetic + L3**	**Diabetic + L4**	**Diabetic + Metformin**	**Diabetic Control**	**Normal Control**
d (g/day/rat)	27.71 ± 3.36^{bcde}	25.40 ± 2.31^{bc}	25.08 ± 1.78^{bc}	25.59 ± 1.85^{bcd}	23.82 ± 1.54^{b}	29.25 ± 5.87^{bcdef}	21.78 ± 1.48^{a}
ter (ml/day/rat)	27.07 ± 3.86^{b}	29.61 ± 4.06^{bcd}	28.01 ± 2.23^{bc}	27.01 ± 2.45^{b}	27.73 ± 2.97^{bc}	32.64 ± 5.19^{bcde}	23.44 ± 1.78^{a}

Values are Mean ± SD of 28 determinations. Values with different superscript across the rows are significantly different ($P < 0.05$)

L1 = Diethyl ether leaves subfraction, L2 = Diethyl ether/chloroform leaves subfraction, L3 =chloroform leaves subfraction, L4 = Methanol leaves subfraction pyruvate kinase activities enhanced significantly (*P<0.05*) compared to the diabetic rats groups that rec

4.10.6 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on glycogen metabolic enzymes in diabetic rats

Activities of glycogen synthase (GS) and phosphorylase (GP) assayed in liver tissues of streptozotocin-induced diabetic rats groups received diethyl ether, diethyl ether/chloroform, chloroform and methanol subfractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves is presented in Table 4.15. Methanol leaves sub-fraction (L4) significantly (P<0.05) suppressed phosphorylase activity (from 2.56±0.27 to 1.02±0.29 U/min/mg protein) but enhanced glycogen synthase (from 0.10±0.00 to 0.28±0.04 U/min/mg protein) compared to the diabetic rats groups that were treated with other subfractions.

4.10.7 Effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca*leaves on gluconeogenesis enzymes in diabetic rats

The activities of glyconeogenic enzymes assayed in diabetic untreated and treated rats groups withmetformin and subfractions of diethyl ether, diethyl ether/chloroform, chloroform and methanol derived from ALF are presented in Table 4.16. Methanol leaves subfraction (L4) significantly (*P<0.05*) suppressed; fructose-1,6-bisphosphatase (from 3.20±0.28 to 0.77±0.08 U/min/ µmole P_i liberated), glucose-6-phosphatase (from 1.90±0.15 to 0.24±0.04 U/min/µmole P_i liberated) and phosphenolpyruvate carboxyl kinase (0.95±0.07 U/min/mg Protein) compared to diabetic rats groups that were treated withother subfractions.

Table 4.14 Effect of Sub-Fractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaveson Glycolytic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

	Animal Grouping						
	Diabetic + L1	**Diabetic + L2**	**Diabetic + L3**	**Diabetic + L4**	**Diabetic + Metformin**	**Diabetic Control**	**Normal Control**
Glucokinase (U/min/mg Protein)	2.77 ± 0.29^{bc}	2.37 ± 0.71^{b}	3.01 ± 0.32^{bcd}	3.27 ± 0.09^{bcd}	5.25 ± 0.07^{bcde}	1.47 ± 0.06^{a}	5.61 ± 0.09^{bcde}
Phosphofructokinase (U/min/mg Protein)	4.49 ± 0.10^{bc}	3.63 ± 0.13^{b}	5.44 ± 0.26^{bcd}	5.44 ± 0.19^{bcd}	6.46 ± 0.64^{bcde}	3.09 ± 0.10^{a}	7.08 ± 0.09^{bcdef}
Pyruvate Kinase (U/min/mg Protein) $x10^{-2}$	1.30 ± 0.04^{b}	1.29 ± 0.02^{b}	1.89 ± 0.18^{bc}	2.19 ± 0.13^{bcd}	2.41 ± 0.05^{bcde}	0.96 ± 0.16^{a}	2.35 ± 0.03^{bcde}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

L1 = Diethyl ether leaves subfraction, L2 = Diethyl ether/chloroform leaves subfraction, L3 =chloroform leaves subfraction,

L4 = Methanol leaves subfraction

Table 4.15Effect of Sub-Fractions Derived from Ethanol-Aqueous Fraction of *Balanites aegyptiaca* Leaveson Glycogen Metabolic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

	Animal Grouping						
	Diabetic + L1	**Diabetic + L2**	**Diabetic + L3**	**Diabetic + L4**	**Diabetic + Metformin**	**Diabetic Control**	**Normal Control**
Glycogen Phosphorylase (U/min/mg Protein)	1.98±0.24^{bcd}	2.17±0.48^{bcd}	1.76±0.23^{bc}	1.02±0.29^{b}	0.96±0.07^{b}	2.56±0.27^{bcde}	0.19±0.08^{a}
GlycogenSynthase (U/min/mg Protein)	0.19±0.01^{b}	0.15±0.03^{b}	0.23±0.01^{bc}	0.28±0.04^{bcd}	0.38±0.01^{bcde}	0.10±0.00^{a}	0.35±0.05^{bcde}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction, **L3** =chloroform leaves subfraction,

L4 = Methanol leavessubfraction

Table 4.16 Effect of Sub-Fractions Derived from Ethanol Aqueous-Fraction of *Balanites aegyptiaca* Leaveson Gluconeogenic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

	Animal Grouping						
	Diabetic + L1	**Diabetic + L2**	**Diabetic + L3**	**Diabetic + L4**	**Diabetic + Metformin**	**Diabetic Control**	**Normal Control**
Glucose 6-Phosphatase (U/min/µmole P_i liberated)	1.54 ± 0.08^{bcde}	1.39 ± 0.12^{bcd}	0.68 ± 0.16^{bc}	0.28 ± 0.05^{a}	0.24 ± 0.04^{a}	1.90 ± 0.15^{bcdef}	0.15 ± 0.03^{a}
Fructose-1,6-Bis-Phosphatase (U/min/µmole P_i liberated)	2.15 ± 0.09^{bcd}	1.62 ± 0.24^{bc}	1.48 ± 0.08^{bc}	0.77 ± 0.08^{b}	0.38 ± 0.04^{a}	3.20 ± 0.28^{bcde}	0.40 ± 0.02^{a}
Phosphoenol-pyruvate carboxylkinase (U/min/mg Protein x 10^{-1})	2.09 ± 0.04^{bcd}	1.88 ± 0.18^{bc}	1.79 ± 0.08^{bc}	0.95 ± 0.03^{b}	0.15 ± 0.07^{a}	2.03 ± 0.17^{bcd}	0.09 ± 0.02^{a}

Values are Mean ± SD of 5 determinations.Values with different superscript across the rows are significantly different (*P<0.05*)

L1 = Diethyl ether leaves subfraction, **L2** = Diethyl ether/chloroform leaves subfraction, **L3**=chloroform leavessubfraction,

L4 = Methanol leavessubfraction

4.11Compounds in Extract of *Balanites aegyptiaca* Leaves

4.11.3 FTIR spectrum of subfractions of *Balanites aegyptiaca leaves*

The FTIR spectrum of methanol sub-fraction derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves showed major absorption at 3253 cm^{-1} which is an indicative of hydroxyl function while absorption between 2016 and 2154 cm^{-1} were considered as C-H (stretch) function. The other absorption include 1558 cm^{-1} which is indicative of C=C from aromatic ring and absorption at 1074 cm^{-1} is due to C–O. Absorption at 1401 cm^{-1} is C-H (bending) as presented in Table 4.16.

4.11.2 Gas chromatograph-mass spectroscopy of B*alanites aegyptiaca* leaves

Results of GC-MS analysis of potent extract-subfractions; methanol (L4) subfraction derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves showed 78 peaks (appendix xviii) where four peaks indicated phenolic compounds based on the National Institute Science and Technology (NIST) library. Given below are the peaks and names of compounds identified (Table 4.17).

S/No:	**Methanol Leaves Subfraction (L4)**	
	Frequencies (cm^{-1})	**Bonds**
1	3257	Hydroxyl group (O-H)
2	2016-2154	C-H (Stretch)
3	1558	C=C
4	1401	C-H (bending)

Table 4.17 FT-IR Spectroscopy of *Balanites aegyptiaca* Leaves Extract-Fraction

5	1075	C-O

Table 4.18 Phytochemicals Identified in Methanol Leaves Subfraction (L4) Derived from Ethanol Aqueous-Fraction of *Balanites aegyptiaca* Leaves by GC-MS Analysis (matching library of ≥80 %)

Peaks	**Retention Time (Min)**	**Peak Area in %**	**Name of Compound**	**Aqual Factor**
Aliphatic Aldehydes				
36	13.929	1.37	7-hexadecenal	95
37	16.364	3.08	9-octadecenal	95
61	24.538	0.81	2-hydroxy-9,17-octadecenienal	94
Fatty Acids				
64	20.927	7.27	hexadecenoic acid	80
66	22.463	8.67	oleic acid	86
68	22.774	5.52	11-octadecenoic acid	99
74	24.666	9.82	glycidyl palmitate	95
Phenolic Compounds				
27	13.385	0.04	2-methoxy-4-vinylphenol	95
29	13.922	0.12	2,6-dimethoxyphenol	83
30	14.003	0.20	2-methoxy-3(-2-propenyl)-phenol	98
35	15.362	0.12	2-methoxy-4-(1-propenyl)-phenol	90
Others				
6	3.253	0.43	pyridine	83
19	8.576	0.14	benzyl alcohol	97
26	11.433	0.05	methyl salicylate	95
46	16.375	0.57	3-eicosene	91
48	16.741	0.16	2-methyl-Z,Z, 13-octadecadienol	90
49	16.910	0.60	2-methyl-2,5-dimethoxybenzaldehyde	80
50	17.134	0.05	cyclopropaneoctanal, 2-octyl	97

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

Balanites aegyptiaca has been reported as antidiabetic medicinal plant which has been studied on both diabetic mice and rats (Mansour and Newairy, 2000; Motaal *et al*., 2012) where the fruit and seedwere reported as useful source of hypoglycemic remedy for the management of diabetes mellitus (Samir *et al*., 2003; George*et al*., 2006; Eman-Helal*et al*., 2013). Compounds like saponins (George*et al*., 2006), rutin (Motaal *et al*., 2012) as well as vanillic and syringic acids (Al-Malki *et al*., 2015) werepostulated as the active compoundsin the plant fruit extract while isorhamnetin 3-rutinoside, 3-robinobioside, quercetin-3-glucoside, aglycones quercetin was reported in the seed kernel (Shafik *et al*., 2016).

Studies have postulated that *Balanites aegyptiaca*fruit and seeds extractscould stimulate insulin secretion (Samir *et al*., 2003), inhibit intestinal α-amylase activity *(*Gad *et al*., 2006), increased muscle basal glucose uptake (Motaal *et al*/. 2012) as well as antioxidant activity (Al-Malki *et al*., 2015) to lowered blood glucose in diabetic-induced animals, which might have been possible due to the variety of biologically active chemicals content of the plnat (*Balanites aegyptiaca)* (Salwa and Hadidi, 1988; Sarker *et al*., 2000; Motaal *et al*., 2012).

Balanites aegyptiaca leaves and stem-bark are used by traditional medicine practitioners in the management of diabetes mellitus and some ailments (Chothani and Vegahasiya, 2011; Abubakar *et al*., 2017),it was also reported that they contain variety of biologically active compounds (Salwa and Hadidi, 1988; Sarker *et al*., 2000; Abdulhamid and Sani, 2016). However, very few or no study has been conducted on their effects as antidiabetic agents. In this study,antihyperglycemic potential of extracts from*Balanites aegyptiaca* leaves and stem-bark alongside the fruit-mesocarp wereassessed on the activities of glucose metabolic enzymes in streptozotocin induced diabetic rats as a step toward identifyinghidden potent compounds and possible mode of actions.A similar study has been conducted by Motaal *et al*(2012)which led to the identification of rutin as the active antidiabetic compounds from the extract-fractions of *Balanites aegyptiaca* fruit.

Normalcy and insignificant changes in parameters observed following the oraltoxi-city study of the ethanol extract-fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit mesocarp at concentrationsup to 4000 mg/kg body weightof rat signified safety of the plant extracts. It was reported that some medicinal plants extract or preparation has less orno toxic effects (Newman and Cragg 2007; Chothani and Vegahasiya, 2011) which may be incline to this plant (*Balanites aegyptiaca*).

Different plants have various protective and therapeutic effects which are essential in prevention of diseases and maintain a state of well being. The medicinal value of plants lies in some chemical substances known as phytochemicals that have a definite physiological action on the human body (Edeoga*et al*., 2005; Momo *et al*., 2011). Some important phytochemicals of plants are alkaloids, tannins, saponins, terpenoids, steroids, glycosides, flavanoids and phenolics (Sarker *et al*., 2000; Momo *et al*., 2011). Presence of flavonoids and phenolics in ethanol extract-fractions of *Balanites aegyptiaca* parts suggested their usefulness in disease prevention. Differences in yields of the extract-fractions of *Balanites aegyptiaca* parts may be due to differences in solubilities and polarities of the extracted compounds. High phenolic compounds in the plantextract attest to the fact that polar solvents promote dissolution of phenolics (Mota *et al*., 2008).

The ultimate target of antidiabetic therapies are plasma glucose homeostatic regulation and amelioration of metabolic derangement (Hers, 1999; Nordlie *et al*., 1999). The ability of the ethanol extract-fractionsof*Balanitesaegyptiaca* partsto lowered blood glucose in glucose loaded rats whichwas further substantiated by their improved glucose tolerance in diabetic-induced rats suggests that the plant's ethanol extract-fractions contain compounds that have the capacity to correct impaired glucose tolerance in diabetes mellitus, hence exhibit antidiabetic effect. Khathi *et al* (2013) have reported similar event using*Syzigium aromaticum*extract in streptozotocin-induced diabetic rats following an oral glucose tolerance test.

Type 1 diabetes mellitus is characterized by severe loss of body weight that may result from relative or absolute deficiency of insulin due to defective β- cells (Bastaki, 2005).A significant decrease in body weight of untreated STZ-induced diabetic rats was observed compared to normal control and diabetic treated rats. The loss of weight in STZ-induced diabetic rats mightbe accompanied by increased breakdown of muscle proteins (for provision of gluconeogenic amino acids) as was suggested by Bastaki (2005) which indicate that degradation of structural protein is a contributing factor towards weight loss. Continuous treatments of STZ-induced diabetic rats with the fractionsof ethanolic extracts of *Balanite aegyptiaca*parts significantly preventedbody weight loss.

Insulin is a potent inhibitor of lipolysis. During diabetes mellitus, activity of lipase enzyme increases lipolysis and release more free fatty acids in the circulation because of lack of insulin (Karthikesan *et al*., 2010). Increase in fatty acid concentration in turn increases the beta-oxidation of fatty acids by increasing the activity of HMG-CoA reductase for production of more cholesterol (Prince and Kannan, 2006; Rydgren and Sandler, 2009). Insulin also increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes mellitus causes hypercho-lesterolemia (Karthikesan *et al*., 2010).

In this study, high levels of cholesterol in diabetic rats was observed, however, STZ induced diabetic rats when treated with the fractions of ethanolic extract of *Balanites aegyptiaca*parts significantly reduced the serum cholesterol levels. It has been reported that plant extracts exert their cholesterol lowering effect by decreasing cholesterol absorption from the intestine via binding with bile acids within the intestine and increasing bile acids excretion (Aderibagbe *et al*., 1999). Significant decrease in serum cholesterol observed in diabetic rats received plant extract-fractions in our study might in part agreed with the above observation. In another dimension, one could suggest the inhibition of HMG-CoA reductase activity by the plant extractssince study has shown an increased HMG-CoA reductase activity in diabetic rats (Rydgren and Sander, 2009).

It has been reported that, hyper-triglyceridemia that characterizes the diabetic state is a consequence of uninhibited actions of pancreatic lipase (Gopalakrishnan and Dhanapal, 2014). From this study, it may be assumed that administration of ethanol extract-fractions of *Balanites aegyptiaca* to diabetic rats might have inhibited the pancreatic lipase activity, which is responsible for the hydrolysis of non-absorbable dietary triglycerides into absorbable monoglycerides and free fatty acids, which, in turn, leads to the decrease of plasma triglycerides level (Unno *et al*., 2005; Sugiyama *et al*., 2007). These findings agreewith the report by Samir *et al* (2003) that aqueous and ethanolic extracts of *Balanites aegyptiaca* fruit were able to decrease serum total cholesterol and triglycerides in STZ-induced diabetic rats.

Increase in the level of serum LDL-cholesterol and VLDL-cholesterol in diabetic untreated rats is an indication of cardiovascular disease development associated with diabetes mellitus. While, reduce serum LDL-cholesterol and VLDL-cholesterol levels with concomitant increase in HDL-cholesterol in the diabetic rats treated with *fractionated ethanol extracts of Balanites aegyptiaca* parts suggest cardioprotective effect which may be due to whole or partly phytochemicals identified. The fractions from ethanol extract may have inhibited hepatic cholesterol biosynthesis as early suggested or stimulate receptor-mediated catabolism of LDL-cholesterol and increase in uptake of LDL from blood by liver as suggested by Khanna *et al* (1996).

It has been reported that glycogen levels in liver tissues decreases as the influx of glucose into the liver is inhibited in the absence of insulin and recovers on insulin treatment (Vats *et al*., 2004). Data from this study showed significant reduction inglycogen content in liver of untreated diabetic rats; decreased glycogen content may result from insulin deficiency leading to a decreasedinflux of glucose in liver and/or decreased activity of glycogen synthase in the liver. Increased glycogen content in rats that received extract of *Balanites aegyptiaca*might probably be due to reactivation of glycogen synthase.Increased hepatic glycogen content due to activation of glycogen synthase by plants extract in diabetic rats has been reported by Jang *et al* (2010) and Ramanchadran and Saravanan(2013).

Proteins such as albumin, globulin, hemoglobin, collagen, LDL, crystalline proteins may undergo non-enzymatic glycation when they are exposed to excess glucose in hyperglycemic state (Wu and Monnier, 2003). Carbonyl group of reducing sugars (e.g., glucose etc.) reacts covalently with amino group of lysine, and arginine to form Amadori rearrangement products. The best know Amadori products are glycated hemoglobin (HbA_{1c}) and fructosamine (fructolysine) (Jakus and Rietbrock, 2004).

Glycated hemoglobin (HbA_{1c}) is generally considered to be a useful index of long term glycemic control of diabetes mellitusbecause it reflects **blood glucose** levels over a period of previous 2-3 months (Phillipou *et al.,* 1988). However, measurement of glycated hemoglobin may not prove useful screening tool as this test does not reflect short-term glycemic changes in blood due to its longer life span (approximately 120 days). Thus, glycated hemoglobin may not depict the exact picture of **blood glucose** fluctuation in short duration studies like this present one. In contrast to this, measurement of glycated plasma proteins known as fructosamine could reveal the status of glycemic control over a period of 2-3 weeks due to their shorter life span (approximately 17-20 days).For this reason, frutosamine was selected as the best parameter to measure in order to access the glycemic changes considering the study duration.

Low level of fructosamine in diabetic treated rats may be due to the restoration of blood glucose levels during the treatment or inhibition of glycationby *Balanites aegyptiaca*extracts. Singh*et al* (2016) have reported inhibition of glycation by *eugenol*, a phenolic compound from plant.Decrease in albumin may be due to albuminuria which are important clinical markers of diabetic nephropathy or glycation (Mauer *et al*., 2007, Qusti *et al*., 2015) and mightalso be due to increased protein catabolism (Almdal and Vilstrup, 2005). Treated diabetic rats with the ethanol extract-fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-meesocarp improved albumin levels. This may be due to the restoration of blood glucose levels by the plant extracts thereby reducing the intensity of protein glycation in the diabetic animals (Srinivasan *et al*., 2014).

Elevation of serum aminotransferases (ALT and AST) activities in diabetic untreated rats in this study indicated an excessive release of such enzymes from the liver cells into the blood circulation. It was reported that there is an inverse relationship between the liver activity and the level of enzymes in serum (Awadallah and El-Dessouky, 1977). This may be consistent with their greater need for gluconeogenesis substrates or may reflect damage of the hepatic cells due to hepatotoxic effect of streptozotocin (Kohl *et al.*, 2013). Diabetic rats treated with fractions from ethanol extract of *Balanites aegyptiaca* parts recorded a decrease in the AST enzyme activity suggesting inhibitory effect of the extract-fractions or from improved liver functions resulted from return gluconeogenesis process towards their normal levels due to the extract-fractions insulinogenic effect as was reported by Hough *et al* (1981) who indicated that, enzymes activity were completely normalized following insulin administration.

However, elevated activity of ALT may suggest failure to prevented the leakage of liver enzymes by *Balanites aegyptiaca* ethanol extract-fractions due to their inability in membrane stabilizing activity since plants extract were reported to have prevented leakage of intracellular enzymes via membrane stabilizing effects (Gopalakrishnan and Dhanapal, 2014).

Decreased PCV levels in diabetic rats is a result of RBC's haemolysis (Nadro and Samson, 2014). Increased lipid peroxidation in the RBC's is due to an inhibition or changing the activity of non enzymatic and/or enzymatic components of the oxidative system (reduced glutathione (GSH), Superoxide Dismutase (SOD) and catalase (CAT) activities (Udoh *et al.*, 2007). Improved PCV level in this study indicates that ethanol extract-fractions of *Balanites aegyptiaca* parts contain compound(s) that has the ability to prevent lipid peroxidation or mop up free radicals. Nadro and Samson (2014) have reported significant protective effect of *Balanites aegyptiaca* kernel cake on PCV levels in alloxan-induced diabetic rats.

The increased food and water intake by diabetic rats maybe due to chronic reduction

in glucose utilization bythe cells and considerable loss of glucose in the urine.It

has been reported that failure of the kidneyto reabsorb glucose in diabetic state resultedin severe loss of renal glucose (Raju and Raju, 2010). Loss of renal glucose is accompanied by loss of water and some electrolytes. Continuoes loss of large volume of water in the diabetic rats might have stimulatedthirst mechanism hence the increased fluid intake bythese animals. Glucosuria and defects in glucose utilization in diabetes mellitus might have increasedthe demand for food. Administration of *Balanites aegptiaca*extract improved glycemic control which prevented excess food and water intake in diabetic treated rats.*Balanites aegptiaca* extract might haveprevented excess food and water intake in diabetic treated rats viaimproved glycemic control. Srinivasan *et al* (2014) have reported that *eugenol*; a phenolic compound argument desired for food and water intake under diabetic condition via glycemic control.

Liver is an insulin-sensitive tissue and plays a major role in glucose metabolism by regulating the interaction between glucose utilization and production (Bhavapriya and Govidasamy, 2000). A partial or total deficiency of insulin causes derangement in glucose metabolism that decreases the activities of glycolytic enzymes causing impaired peripheral glucose utilization while promoting gluconeogenesis (Srinivasan *et al*., 2014). Part of the objective of the study is to determine whether*Balanites aegyptiaca* modulates enzymes involved in glucose and glycogen metabolism.

Activities of glucokinase, phosphofructokinase and pyruvate kinase hasbeen shown

to be very sensitive signs of the glycolytic pathway and these are decreased in the liver of diabetic state (Murphy and Anderson, 1974). Reduced activities of these enzymes in this study are consistent with other studies on glycolytic enzymes (Latha and Pari, 2003; Soliman *et al.*, 2013).Reduced activities of these enzymes in the diabetic rats may be due to lack of insulin and the reason for the diminished utilization of glucose in the system and increased blood glucose levels (Gardiner *et al*., 2007).Insulin influences the intracellular utilization of glucose bypromoting glycolysis byincreasing the activity and amount of glycolytic enzymes (Latha and Pari, 2003; Silva *et al*., 2010). Administration of fractions of the ethanolic extractof *Balanites aegyptiaca*induced significant increase in the activities of glycolytic enzymes supporting the notion that part of the therapeutic potential of several putative antidiabetic plants can involve the modulation of enzymes in carbohydrate metabolism (Nachar *et al*., 2013; Deepak *et al*., 2014).

Specifically, the activity of glucokinase (GK), a rate limiting enzyme involved in the hepatic storage and utilization of glucose showed a significant change in activityin this study as reported by Smith *et al* (2005) and Agius (2007). The activity of hepatic GK is reported to be reduced in diabetes mellitus and can be activated by an activator (Priyadarsini *et al* 2012). Plant extracts have been reported to exert antihyperglycemic activityin part by promoting the activity of this enzyme(Pari and Satheesh, 2006; Farsi *et al* 2014).Increased glucokinaseactivity in diabetic rats treated with extracts of *Balanites aegyptiaca*particularly the fruit-mesocarp might have improve glycolysis leading to the reduction in blood glucose. Shafik *et al* (2016) have shown that extract of *Balanites aegyptiaca* seed-kernel promotes the activity of hexokinase, suggesting glycolysis stimulatoryeffect of the plant.

However, it was reported that the first irreversible reaction unique to glycolytic pathwayis the phosphorylation of fructose 6-phosphate to fructose-1,6-bisphosphate which is catalyzed by the phosphofructokinase.Theenzyme which catalyzes the committed step in metabolic sequence is the most important control element in the pathway (Berg *et al*., 2002; Satyanarayana and Chakrapani, 2006). In another dimension,according to Berg *et al* (2002), the glucose 6-phosphate produced by glucokinase is not solely glycolytic intermediatesince, it could be converted to glycogen or oxidized by the pentose phosphate pathway to produce NADPH indicating that glucokinase does not catalyzed committed step in the glycolytic pathway. Increased phosphofructokinase activity in the diabetic rats treated with fractions of the ethanolic extracts of *Balanites aegyptiaca* parts particularly by the leaves extract suggests that it contain compounds thatregulate glycolysis.

Glucose-6-phosphate dehydrogenase is the first rate limiting enzyme of the pentose phosphate pathway which results in the production of ribose-5-phosphate and the reducing equivalent NADPH (Xu, *et al*., 2005). The study observed suppressed activity of G6PDH in liver of STZ-diabetic rats similar to reports by Karuna *et al*., (2010) and Saralakumari *et al*., (2013). Decreasedactivity of G6PDH was due to deficiency of insulin, Wagle *et al* (1998) have reported that insulin regulated G6PDH gene for its activity. Diaz-Flores *et al* (2006) have reported that decreased hepatic G6PDH activity depends on the severity of hyperglycemia. Decreased activity of G6PDH indicated low level of NADPH produced by hexose monophosphate pathway (HMP) which is unable to meet the cellular requirement for the enzymes that continuously maintain glutathione (GSH) in its reduced state or accumulation of glucose-6-phosphate which is a potent glycosylating agent that causes GSH depletion and thereby boosts glycation and it may also promote the final step of gluconeogenesis (Jain, 1998).

*Balanites aegyptiaca*extractsadministration increased G6PDH activity in the diabetic treated rats, accompanied by increase in NADPH levels, a product of the HMP pathway. This might suggest that the extracts of *Balanites aegyptiaca*has the capacity to modulate hexose monophosphate pathway for alternative glucose oxidation. In another dimension, according to Atangwho *et al* (2014), glucose oxidation via gluconate pathway is associated with increased glucokinase activity. Therefore it is suggested that activation of G6PDH in the diabetic rats groups particularly that received the fruit extract of *Balanites aegyptiaca* might be a reflec-

tion of the increased activity of their glucokinase.

Gluconeogenesis is a main cause of the elevated hepatic glucose production contributing 50-60 % of the released glucose (He *et al*., 2009). Insulin decreases gluconeogenesis by decreasing the activities of enzymes, such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase (Skim *et al*., 1999; Murray *et al.,* 2003). The rate of gluconeogenesis is regulated by the activity of two rate limiting gluconeogenic enzymes; glucose-6-phosphatase and fructose-1,6-bisphosphatase. G6pase, is a key enzymes in the homeostatic regulation of blood glucose and is critical in providing glucose to other organs during diabetes mellitus(Nordlie and Foster, 2010).

Fructose-1,6-Bisphosphataseiskey regulator enzymes of the hepatic gluconeogenesis and appeares as target for efficient and safe glycemic control in diabetes mellitus (Zhang *et al*., 2010). In this study extract of *Balanites aegyptiaca* suppressed the activities of these enzymes. This findings is inlinewith other studies where several plants extractwere reported to have suppressed the activities of these enzymes in diabetic animals (**Kazeem *et al*., 2013;** Srivastava *et al.* 2014; Kolawole and Akanji2014; **Birudu *et al* 2015**).Shafik *et al* (2016) have reported that extract of *Balanites aegyptiaca* seed-kernel suppressed glucose-6-phosphatase activity.

It has been noted that metformin inhibit hepatic gluconeogenesis to achieved anti-diabetic effect (Meyer*et al*., 2008; Viollet *et al*., 2012). *Balanites aegyptiaca*might have lowered blood glucose in part byinhibition of hepatic gluconeogenesis.Plants like *Eugenea jambolana (*Sharma *et al*., 2011*)*, ***Centaurea bruguie-rana ssp. Belangerana***(Khanavi*et al*., 2012), and *Juglans regia* (Khoramdelazad *et al.* 2015) have been reported to inhibit hepatic gluconeogenesis as part of their anti-diabetic mechanism.

Glycogen synthase (GS) catalyzes the rate limiting step in glycogen syhthesis and is thus responsible for the storage of glucose as glycogen in the liver. Fractions of ethanolic extracts of *Balanites aegyptiaca*parts appear to have little effect on glycogen synthase activity but suppressed phosphorylase activity significantlyin the diabetic treated rats. This is supported by the relativechange in glycogen content in liver of the diabetic treated rats. Some plants extract have been reported to regulatedglycogen enzymes leading toincreased hepatic glycogen content (Jang *et al*., 2010; Ramachadran and Saravanan, 2013). According to Gutierrez (2013), activation of glycogen synthase by plant suggested insulinogenic character; going by this statement one may propose that *Balanites aegyptiaca* contains component that exhibits insulin like effect.

The present study found*Balanites aegyptiaca* leavesand fruit aqueous fractionsas more comparable to Metformin in terms of fasting blood glucose reduction, reverse lipid profile and regulating activities of key regulatory enzymes (phosphofructokinase, fructose-1,6-bisphophatase, glycogen phosphorylase, glycogen synthase and glucose 6-phosphodehydrogenase) involved in various glucose metabolic pathways.The study found that, the leaves aqueous fraction was more comparable to Metformin in term of glucose enzymes regulation.In addition,antidiabetic effect of *Balanites aegyptiaca*leaveshas not been study sufficiently despite its use by the traditional medicine practitioners for the management of diabetes mellitus in Nigeria (Abubakar *et al*., 2017).Guided by these facts, the aqueous leaves fraction (ALF) being one of the most potentwas further fractionated and evaluated to facilitate the identification of hidden potent compounds.

The study showed methanol (L4) leaves subfraction exhibited activities in a similar

manner as the ALF where spectra elucidation of the methanol (L4) subfractionled to the identification of several compounds including some phenolics. Al-malki *et al* (2015) has early reported some phenolics; vanillic and syringic compoundsin *Balanites aegyptiaca* fruit and this is an indicative that phenolic compounds form part of the antidiabetic properties of *Balanites aegyptiaca.*Recently, Gawade and Farooqui (2018) identified a phenolic compound from leaves extract of this plant.

The phenolic compounds; 4-vinylguaiacol, syringol, eugenol, and transisoeugenol identified in *Balanites aegyptiaca* leaves extract may accounted for the observed biological activities in the study.It has been reported that phenolics and flavonoids are plant chemicals that accounted for the majorpharmacological activities (Mota *et al*., 2008). Absence of most of the identified phenolic compounds from extract of the *Balanites aegyptiaca*fruit-mesocarp implies variation in distribution of the plant's chemical compounds and this may account for the differences in biological activities exhibited by the plant parts.Literature reviewhave reported;4-

vinylguaiacol, syringol, eugenol and transisoeugenol from some medicinal plantsextract (Purwantiningsih *et al*., 2011; Dandekar *et al*., 2015), and were postulated to be responsible for antioxidant, anti-inflammation,anticancer, antidiabetic activity exerted(Jeong and Jeong, 2010; Suttiarporn *et al*., 2015; Singh *et al*., 2016).

A study has reported that consumption of foods and drinks that contain high phenolic contents is associated with prevention of diabetes mellitus(Bahadoran*et al*., 2013). Eugenol,a phenolic compoundisolated from Clove plant has been reported to influence the activities of enzymes involved in glucose metabolism contributingto the maintenance of normalglycemia (Srinivasan *et al*., 2014; Jeong*et al*., 2014).Singh *et al* (2016) have also reported antidiabetic effect of *eugenol*isolated from *Ocimum gratissimum*leaves as effective alpha-glucosidase and glycation inhibitors. A phenolic compound ‘caffeic acid’ was reported to have improved glucose metabolism in diabetic rats’ via inhibition of gluconeogenic enzymes (Celik *et al* (2009).Kim *et al* (2016) have reported antidiabetic activityof isoeugenol, a plant phenolic compoundbyincreasing glucose upake in skeletal muscle. Inline withthe above reports, *Balanies aegyptiaca*leavesextractmay have improved glycemic control by reversing the activities of enzymes involved in glucose and glycogen metabolismswhich may be attributed bysomeor allthe phenoliccompounds identified.

CHAPTER SIX

6.0SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The findings of this study is summarized as follows;

Ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp are relatively safe with LD_{50} above 4000 mg/kg body weight of wistar albino rats.

Phenolics and flavonoids content varied in the ethyl acetate and aqueous fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp. High phenolics content was measured in the aqueous fractions (aqueous fruit-mesocarp fraction, 77.74±1.68 x 10^{-2} mg/g GAE)whereas flavonoids was high in the ethyl acetae fractions (ethyl acetate stem-bark fraction, 11.41±1.24 x 10^{-2} mg/g QE).

Fractionated ethanol extracts of *Balanites aegyptiaca* parts exerted antihyperglycemic and antilipidemic effects in STZ-induced diabetic rats. The fruit-mesocarp and leaves aqueous fractions were more comparable in lowering fasting blood glucose by 18.61 % and 15.87 % when compared to 24.62 % by metformin.

Fractionated ethanol extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarpsignificantly reversedthe activities of key regulatory enzymes of glucose metabolism. In comparable of the glucose enzymes regulatory effect, the aqueous leaves fraction was more comparable to metformin, it significantly suppressed the activities of fructose 1,6-bisphosphatase (from 2.19±0.25 to 1.32±0.06 U/min/µmole Pi liberated) and glycogen phosphorylase (from 3.82±0.21to 2.76±0.02 U/min/mg protein)but enhanced phosphofructokinase (from 2.06±0.07to 2.52±0.03 U/min/mg protein)and glycogen synthase (from 9.41±0.34 x $10^{-2}$to 14.45±0.16 x 10^{-2} U/min/mg protein) whereas theaqueous fruit-mesocarp fraction (AFF) significantly enhanced glucose 6-phosphodehydrogenase (from 1.40±0.02 to 2.10±0.01 U/min/mg protein) and glucokinase (from 2.22±0.02 to 3.58±0.05 U/min/mg protein).

Subfractions (diethyl ether, diethy ether/chloroform, chloroform and methanol) from aqueous leaves fraction (ALF) of *Balanites aegyptiaca* exhibited antihyperglycemic, antilipidemic and reversed the activities of glucose metabolizing enzymes.Methanol (L4) subfractionwas more comparable to the ALF and Metformin; itlowered blood glucose level by 19.18 % compared to 22.93 % fasting blood glucose reduction by Metformin. L4suppressed glycogen phosphorylase (from 2.56±0.27to 1.02±0.29 U/min/mg protein) compared to 0.96±0.07 U/min/mg protein by Metformin, whereas fructose 1,6-bisphosphatase (from 3.20±0.28to 0.77±0.08 U/min/µmole Pi liberated) compared to 0.38±0.04 U/min/µmole Pi liberated by Metformin. It also enhanced glycogen synthase activity (from 0.10±0.00 to 0.28±0.04 U/min/mg protein) compared to 0.38±0.01 by Metformin whereas, phosphofructokinase (from 3.09±0.10to 5.44±0.19 U/min/mg protein) compared to 6.46±0.64 U/min/mg protein by metformin.

The possible active ingredeint of the methanol subfractionwere4-vinylguaiacol, syringol, eugenol and transisoeugenol.

6.3 Conclusion

Based on the findings of this study, it is concluded that;

I. Ethanol extract-fractionsof*Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp are safe in wistar albino rats.

II. *Balanites aegyptiaca*is rich with phenolics and flavonoids; the two plants compounds that accounted for the majority ofpharmacological activities.

III. Fractionated ethanol extracts of *Balanites aegyptiaca*parts exerted antihyperglycemic, antihyperlipidemic and reversed diabetes induced alterations in enzymes related to carbohydrate metabolism.

IV. The leaves extract of *Balanites aegyptiaca*exert antidiabetic activity as comparable to the fruit-mesocarp. The leaves extractseem to contain compounds that likely reversesthe activities of enzymes in both glucose catabolism and anabolism whereas, the fruit-mesocarp extract enhanced enzymes of glucose catabolism.

V. Phenolic compounds identified in the leaves extract of*Balanites aegyptiaca*may likely be the bioactive molecules.

6.2Recommendations

I. Having partially purified extract from *Balanites aegyptiaca* leaves and identified some compounds (phenolics) that might have contributed toward achieving antihyperglycemic activity, there is the need to isolate these compounds and compare their antihyperglycemic effect with available synthetic ones.

II. Investigation into the molecular mechanism/gene expressions for glucose metabolic enzymes involved in the remedial effect of *Balanitesaegyptiaca*leaves extract on diabetes mellitusis recommended to further understand the mechanism of antihyperglycemic action.

III. Different extraction techniques should be employed to further isolate and characterize active compounds from *Balanites aegyptiaca* leaves.

6.3 Study Contribution to Knowledge

I. The study identified leaves of*Balanites aegyptiaca* as one of the partwith potential antidiabetic activity beside the fruit-mesocarp that has earlier been reported.The leaves extractlowered fasting blood glucose by 15.87 %, fruit-mesocarp(18.61 %)and standard antidiabetic drug; metformin (24.62 %).

II. The study was able to identify that*Balanites aegyptiaca*leaves extract reversed the activities of enzymes involved in glucose and glycogen metabolisms to probably exert antihyperglycemic activity. It suppressed fructose 1,6-bisphosphatase (from 2.19±0.25 to 1.32±0.06 U/min/µmole Pi liberated) and glycogen phosphorylase (from 3.82±0.21 to 2.76±0.02 U/min/mg protein) but enhancedphosphofructokinase (from 2.06±0.07to 2.52±0.03 U/min/mg protein) and glycogen synthase (from 9.41±0.34 x 10^{-2} to 14.45±0.16 x 10^{-2} U/min/mg protein).

III. The study identifiedsomephenolic compounds; springol (0.12 %), isoeugenol (0.12 %), eugenol (0.23 %), 4-vinyl guaiacol (0.67 %)from*Balanites aegyptiaca*leavesextract that may likely be the active principle.

IV. The plant leaves with the bioactive compounds identified in this study is a step toward finding safer and effective pharmaceutical ingredient for development of novel antidiabetic drug.

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APPENDICES

Appendix I: Values for Hypoglycemic Effect of Extract-fractions of *Balanites aegyptiaca*Parts on Normoglycemia in Fasted Animals

Extracts	**Sampling Time (minutes)**				
ALF	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
	104	95	83	78	73
	98	90	86	84	80
	99	100	92	88	74
ELF					
	79	74	70	68	65
	88	82	78	76	72
	85	80	74	70	67
AFF	89	80	87	83	77
	77	72	68	65	60
	85	81	74	71	68
EFF	92	90	87	80	77
	99	94	90	84	79
	87	87	86	84	80
ASF	95	91	85	71	80
	90	84	77	70	74
	86	80	72	68	60
ESF	79	80	85	77	75
	88	84	77	74	73
	85	84	72	70	69
Metformin	100	74	64	53	42

99	91	83	68	54
74	67	62	55	46

Appendix II: Values for Effect of *Balanites aegyptiaca*Parts Extracts-Fractions on Oral Glucose Tolerance in Normoglycemic Animals

Extracts	**Sampling Time (minutes)**				
ALF	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
	81	86	80	71	69
	79	84	81	76	73
	83	90	85	80	78
ELF					
	74	99	100	88	86
	62	95	90	77	70
	67	90	98	93	90
AFF					
	93	113	133	115	80
	99	122	130	117	86
	99	120	128	120	79
EFF					
	91	110	118	104	107
	92	115	104	107	104
	102	120	110	108	100
ASF					
	99	112	106	94	87
	100	117	109	99	93
	96	115	100	97	90
ESF					
	80	102	110	95	100
	71	99	103	97	95
	79	100	114	101	87
Metformin					
	88	104	96	68	67
	80	100	98	80	75

	89	112	108	73	70
Vehicle(DMSO)					
	80	115	120	127	132
	81	117	123	126	126
	86	120	128	126	130

Appendix III. Values for Effect of *Balanites aegyptiaca's*Parts Extracts-Fractions on Oral Glucose Loaded Test in STZ DiabeticAnimals

Extracts	**Sampling Time (minutes)**			
ALF	**0 min**	**30 min**	**60 min**	**120 min**
	323	320	289	240
	309	302	298	227
	294	298	266	238
ELF	342	255	224	209
	313	300	286	223
	319	247	221	200
AFF	325	300	269	300
	330	323	300	311
	310	307	297	281
EFF	264	275	239	207
	284	300	310	315
	238	260	283	238
ASF	305	317	256	326
	298	269	266	340
	290	300	267	291
ESF	286	300	310	288
	250	272	307	294
	300	317	324	278
Metformin	308	270	265	222
	298	286	253	200

	276	269	241	218
Vehicle (DMSO)	257	300	317	334
	300	315	321	333
	251	286	290	321

Appendix IV. Values for Water and Feed Intakes of Animals Treated with *Balanites aegyptiaca's*Parts Extracts-Fractions

Animals' Groups	**Period (Weeks)**	**Water intake (ml/rat)**	**Feed intake (g/rat)**
Diabetic + ALF	1	17.4	23.86
	2	30.6	29.11
	3	26.49	31.86
	4	20.93	33.51
Diabetic + ELF	1	16.00	14.13
	2	33.21	30.26
	3	32.28	32.1
	4	25.01	30.18
Diabetic + AFF	1	15.89	25.29
	2	32.14	28.69
	3	30.97	25.91
	4	26.4	30.77
Diabetic + EFF	1	15.86	26.00
	2	30.27	27.38
	3	29.2	26.23
	4	27.01	32.36
Diabetic + ASF	1	15.97	25.8
	2	29.14	28.63
	3	30.71	26.29
	4	24.1	29.31
Diabetic control	1	15.8	14.23
	2	30.06	23.83
	3	34.86	25.37
	4	35.43	29.66

Diabetic + metformin	1	15.86	21.14
	2	26.00	25.54
	3	25.14	25.09
	4	18.87	29.71
Normal control	1	18.94	27.00
	2	15.94	35.00
	3	20.09	32.6
	4	15.7	34.34

Appendix V. Hepatic Glucose Metabolic Enzymes of Animals Treated with *Balanites aegyptiaca's*Parts Extracts-Fractions

A. Values of glycolytic and gluconegenic enzymes' activities of the experimental animals

	GK(U/min/ mg Protein)	PFK (U/min/mg Protein)	PK(U/min/ mg Protein)	LDH (U/min/mg Protein)	G6Pase (U/min/μ mol Pi liberated)	F16BPase (U/min/μ mol Pi liberated)	PEPCK (U/min/mg Protein)	PC (U/min/mg Protein)
Diabetic + ALF	2.75	2.51	0.02	0.12	0.12	1.26	0.16	0.03
	2.75	2.52	0.02	0.12	0.13	1.26	0.16	0.03
	2.76	2.56	0.03	0.12	0.14	1.37	0.15	0.03
	2.75	2.52	0.02	0.12	0.14	1.33	0.17	0.03
	2.75	2.48	0.02	0.12	0.15	1.37	0.17	0.02
Diabetic +ELF	2.23	2.32	0.01	0.11	0.15	1.6	0.22	0.01
	2.23	2.33	0.02	0.11	0.14	1.3	0.21	0.01
	2.23	2.32	0.01	0.11	0.15	1.38	0.2	0.01
	2.23	2.33	0.01	0.11	0.16	1.61	0.2	0.01
	2.23	2.35	0.02	0.11	0.15	1.25	0.2	0.01
Diabetic +AFF	3.57	2.32	0.03	0.04	0.16	1.37	0.31	0.07
	3.66	2.38	0.03	0.04	0.17	1.38	0.41	0.07
	3.56	2.38	0.03	0.07	0.16	1.5	0.4	0.07
	3.55	3.01	0.03	0.08	0.17	1.44	0.4	0.07
	3.55	1.41	0.03	0.07	0.16	1.37	0.39	0.07
Diabetic +EFF	3.17	1.3	0.01	0.11	0.25	1.16	0.48	0.04
	3.17	1.27	0.01	0.04	0.25	1.18	0.45	0.04
	3.17	1.24	0.01	0.06	0.25	1.23	0.46	0.04
	3.17	1.22	0.01	0.07	0.25	1.2	0.46	0.04
	2.85	1.22	0.01	0.07	2.5	1.24	0.45	0.04
Diabetic +ASF	3.07	2.73	0.01	0.06	0.21	1.4	0.19	0.02
	3.08	2.57	0.01	0.05	0.27	1.34	0.2	0.02
	3.08	2.73	0.01	0.07	0.28	1.37	0.2	0.02
	3.08	2.89	0.01	0.06	0.27	1.43	0.21	0.02
	2.59	2.73	0.01	0.07	0.26	1.57	0.2	0.02
Diabetic control	2.24	2.11	$0.5*10^{-3}$	0.05	1.35	2.44	1.02	0.11
	2.23	2.09	$0.6*10^{-3}$	0.08	1.46	2.15	0.87	0.12
	2.22	2.09	$0.4*10^{-3}$	0.09	1.45	2.15	0.77	0.12
	2.21	2.09	$0.3*10^{-3}$	0.07	1.46	1.81	0.71	0.12
	2.2	1.93	$0.2*10^{-3}$	0.07	1.48	2.37	0.65	0.12

Diabetic +metformin	2.73	3.33	0.02	0.08	0.1	1.00	0.14	0.03
	2.72	3.33	0.01	0.05	0.1	1.01	0.1	0.03
	2.73	3.34	0.01	0.03	0.13	1.01	0.06	0.03
	2.71	3.34	0.02	0.07	0.11	1.04	0.14	0.03
	2.69	3.36	0.01	0.08	0.14	1.05	0.11	0.03
Normal control	3.54	4.42	0.01	0.07	0.06	1.34	0.09	0.02
	3.54	4.49	0.01	0.04	0.07	1.43	0.1	0.02
	3.53	4.47	0.01	0.03	0.06	1.36	0.1	0.02
	3.53	4.31	0.01	0.05	0.09	1.35	0.09	0.02
	3.53	4.49	0.01	0.03	0.07	1.52	0.08	0.02

B. Values of glycogen and pentose phosphate pathway metabolic enzymes' activities of all experiemental animals

	GP **(U/min/mg Protein)**	GS **(U/min/mg Protein)**	G6PDH **(U/min/mg Protein)**
Diabetic + ALF	2.79	0.14	1.73
	2.75	0.14	1.73
	2.75	0.14	1.74
	2.74	0.15	1.74
	2.74	0.15	1.73
Diabetic +ELF	2.75	0.12	1.6
	2.75	0.12	1.61
	2.73	0.12	1.6
	2.74	0.12	1.61
	2.75	0.12	1.61
Diabetic +AFF	3.75	0.12	2.1
	3.75	0.12	2.11
	3.75	0.12	2.1
	3.74	0.12	2.1
	3.75	0.13	2.1
Diabetic +EFF	3.46	0.12	1.7

	3.46	0.12	1.71
	3.46	0.12	1.69
	3.47	0.12	1.69
	3.45	0.12	1.72
Diabetic +ASF	3.09	0.12	1.3
	3.15	0.13	1.29
	3.06	0.12	1.3
	3.19	0.13	1.29
	3.23	0.12	1.29
Diabetic control	3.68	0.09	1.48
	3.68	0.09	1.45
	4.17	0.1	1.46
	3.69	0.09	1.43
	3.86	0.09	1.42
Diabetic +metformin	2.04	0.15	1.89
	2.03	0.16	1.88
	2.04	0.15	1.89
	2.04	0.16	1.89
	2.03	0.16	1.88
Normal control	2.08	0.29	2.05
	2.07	0.3	2.06
	2.07	0.28	2.06
	2.06	0.3	2.05
	2.07	0.3	2.06

Appendix VI. Values of Liver Parameters of Animals Treated with *Balanites Aegyptiaca*Parts Extracts-Fractions

	Glycogen **(g/g liver)**	Protein **(mg/g liver)**	Albumin **(g/dl)**	Fructosamine **(mmol/L)**	AST **(U/L)**	ALT **(U/L)**	Insulin **(ng/L)**
Diabetic + ALF	18.46	21.49	4.01	0.1	11.5	9.2	0.24
	17.69	21.58	4.01	0.09	12	8.8	0.34
	18.46	21.67	4	0.09	14	9	0.23
	18.08	21.55	3.99	0.09	15	9.2	0.27
	18.85	21.59	3.98	0.05	15	9	0.27
Diabetic +ELF	17.69	21.03	2.43	0.06	12.5	5.2	0.14
	20.77	20.75	2.44	0.07	13	5.4	0.2
	18.08	21.02	2.44	0.07	13.5	5.2	0.32
	17.31	20.66	2.45	0.05	13	5	0.22
	16.92	20.76	2.44	0.08	12	5.2	0.22
Diabetic +AFF	20.77	19.64	4.19	0.07	11.5	6	0.18
	21.15	19.63	3.88	0.06	11.5	6.2	0.35
	20.77	19.61	3.87	0.07	12	6.2	0.45
	20.38	19.6	3.88	0.07	11	5.8	0.33
	20	19.66	3.86	0.07	11.5	6.2	0.33
Diabetic +EFF	15	20.98	4.25	0.06	8.5	7	0.12
	14.62	21.03	4.25	0.06	8.5	5.2	0.11
	14.62	21.08	4.23	0.07	9.5	6	0.12
	14.23	21.12	4.23	0.06	9	5.2	0.16
	14.23	21.09	4.22	0.04	12.5	6.4	0.13
Diabetic +ASF	13.85	20.52	2.57	0.05	22.5	4.2	0.13
	13.85	20.75	2.57	0.04	25.5	4.2	0.17
	13.46	20.53	2.57	0.05	25	4.4	0.12
	14.23	20.7	2.56	0.06	20	5	0.15
	13.46	16.32	2.57	0.06	21	4.4	0.14
Diabetic control	10.77	16.51	2.34	0.26	13	4.2	0.11
	10.38	16.45	2.34	0.26	13	4.2	0.28
	11.15	16.45	2.29	0.27	13.5	4.4	0.34
	10.77	16.49	2.04	0.24	13.5	4.6	0.1
	10.38	20.85	2.04	0.42	13.5	4	0.11
Diabetic +metformin	17.69	24.99	3.59	0.04	12.5	4.2	0.11
	17.69	25	3.6	0.04	13	2.6	0.11
	18.08	24.96	3.59	0.05	14	3.6	0.07
	18.08	25.04	3.59	0.06	12.5	2.2	0.09

	17.31	25.07	3.59	0.05	12.5	3.6	0.1
Normal control	16.15	26.88	4.27	0.03	11.5	3.6	0.40
	15.77	26.94	4.28	0.04	11.5	3.4	0.51
	15.77	26.98	4.28	0.05	12.0	3.4	0.34
	16.15	26.95	4.28	0.04	12.0	3.6	0.51
	15.38	26.97	4.27	0.03	11.5	3.4	0.51

Appendix VII. Values of Lipids Profiles of Animals Treated with *Balanites aegyptiaca* Parts Extracts-Fractions

	Cholesterol **(mg/dL)**	TG **(mg/dL)**	HDL **(mg/dL)**	LDL **(mg/dL)**	VLDL **(mg/dL)**
Diabetic + ALF	170.77	116.09	50.69	137.41	23.22
	172.31	119.13	50.17	138.45	23.83
	175.38	120	49.83	141.42	24
	167.69	123.04	48.96	133.29	24.61
	173.85	121.3	50.35	139.52	24.26
Diabetic +ELF	230.77	180.43	58.68	182.95	36.09
	232.31	193.04	61.11	181.48	38.61
	235.38	189.13	61.28	185.3	37.83
	203.08	184.78	63.02	153.52	36.96
	221.54	179.57	60.24	173.58	35.91
Diabetic +AFF	172.31	140	50	134.31	28
	173.85	140.43	49.83	135.79	28.09
	170.77	142.17	51.39	132.06	28.43
	166.15	141.74	52.08	127.39	28.35
	169.23	137.83	50.87	131.49	27.57
Diabetic +EFF	216.92	144.35	51.74	177.71	28.87
	218.46	144.78	53.82	178.74	28.96
	220	139.57	69.44	178.2	27.91
	212.31	147.39	50.35	172.76	29.48
	221.54	139.57	52.08	183.21	27.91
Diabetic +ASF	212.31	226.52	38.72	159.26	45.3
	210.77	226.96	43.4	156.7	45.39
	209.23	232.61	37.33	155.24	46.52
	213.85	233.91	40.1	159.04	46.78
	215.38	229.13	37.85	161.99	45.83
Diabetic control	233.85	200.43	24.13	188.93	40.09
	235.38	200.87	23.61	190.49	40.17
	232.31	213.48	24.31	184.75	42.7
	227.69	210	35.59	178.57	42
	230.77	210.87	28.47	182.9	42.17
Diabetic +metformin	176.92	99.57	62.33	144.54	19.91
	178.46	103.91	74.48	142.78	20.78
	180	106.96	66.84	145.24	21.39
	183.08	94.78	67.88	150.54	18.96

	184.62	94.35	60.24	153.7	18.87
Normal control					
	75.38	97.83	49.83	45.85	19.57
	73.85	98.7	42.53	45.6	19.74
	76.92	98.7	49.65	47.25	19.74
	81.54	93.04	48.61	53.21	18.61
	73.85	100.87	48.26	44.02	20.17

Appendix VIII. Values for PCV (%) of Animals Treated with *Balanites aegyptiaca* Extracts-Fractions

	Day 0	Day 7	Day 14	Day 21	Day 28
Daibetic +ALF	25	31	37	30	30
	36	42	40	36	32
	43	35	38	29	21
	30	36	40	30	28
	33	35	38	29	27
Mean	**33.4**	**35.8**	**38.6**	**30.8**	**27.6**
SD	6.73052	3.962323	1.34164	2.94957	4.159327
Daibetic +ELF	36	33	33	28	20
	26	22	17	24	25
	31	30	29	25	40
	30	29.5	30	28	27
	29.5	29	27	27	30
Mean	**30.5**	**28.7**	**27.2**	**26.4**	**28.4**
SD	3.605551	4.05586	6.09918	1.81659	7.436397
Daibetic +AFF	30	33	36	35	27
	32	25	26	27	26
	33	27	24	25	25
	30	28	27	25	25
	31	29	26	26	24
Mean	**31.2**	**28.4**	**27.8**	**27.6**	**25.4**
SD	1.30384	2.966479	4.71168	4.21900	1.140175
Daibetic +EFF	28	33	38	45	38
	27	27	35	30	31
	28	31	30	25	26
	29	32	30	33	30
	29	30	29	32	27
Mean	**28.2**	**30.6**	**32.4**	**33**	**30.4**
SD	0.83666	2.302173	3.91152	7.38241	4.722288
Daibetic +ASF					
	35	22	21	26	38
	34	36	25	29	33
	34	33	31	30	30
	33	32	30	28	27
	35	33	31	29	28
Mean	**34.2**	**31.2**	**27.6**	**28.4**	**31.2**

SD	0.83666	5.357238	4.44971	1.516575	4.438468
Daibetic +ESF	29	25	25	-	-
	32	33	32	29	-
	33	18	29	30	-
	33	30	30	-	-
	31	29	27	29	-
Mean	**31.6**	**27**	**28.6**	**29.3333**	**-**
SD	1.67332	5.787918	2.70185	0.57735	
Diabetic Control	32	33	30	36	21
	34	27	30	30	20
	29	30	21	22	19
	26	25	28	33	25
	36	30	37	36	27
Mean	**31.4**	**29**	**29.2**	**31.4**	**22.4**
SD	3.9749	3.082207	5.71839	5.813777	3.435113
Daibetic +Metformin	33	25	25	25	30
	28	33	32	32	34
	36	33	40	39	33
	22	31	39	27	35
	26	37	35	34	33
Mean	**29**	**31.8**	**34.2**	**31.4**	**33**
SD	5.56776	4.38178	6.05805	5.59464	1.870829
Normal Control	33	38	34	31	34
	34	31	38	35	34
	34	25	26	25	37
	31	30	25	36	28
	38	29	29	30	27
Mean	**34**	**30.6**	**30.4**	**31.4**	**32**
SD	2.54951	4.722288	5.50454	4.393177	4.301163

Appendix IX. Values for Polyphenols Content of *Balanites aegyptiaca* Parts Extracts-Fractions

A. Phenolics concentrations

ALF	ELF	AFF	EFF	ASF	ESF	
0.728863	0.612245	0.787172	0.058309	0.553936	0.055394	
0.612245	0.553936	0.758017	0.087464	0.466472	0.06414	
0.641399	0.524781	0.787172	0.049563	0.524781	0.069971	
0.660836	**0.563654**	**0.777454**	**0.065112**	**0.515063**	**0.063168**	**MEAN**
0.06069	0.044534	0.016832	0.019845	0.044534	0.007337	**SD**

B. Flaonoids concentrations

0.035303	0.014965	0.024942	0.053722	0.034919	0.09977	
0.034919	0.014198	0.02571	0.05449	0.039524	0.122026	
0.034536	0.015349	0.026861	0.052571	0.033384	0.120491	
0.034919	**0.014838**	**0.025838**	**0.053594**	**0.035943**	**0.114096**	**MEAN**
0.000384	0.000586	0.000966	0.000966	0.003195	0.01243	**SD**

Appendix X. Values for Fasting Blood Glucose of Diabetic and Non-Diabetic Animals Following Treatment with *Balanites aegyptiaca* Parts Extracts-Fractions

	Day 0	Day 7	Day 14	Day 21	Day 28	
Diabetic +ALF	252	289	243	219	200	
	252	281	267	246	218	
	252	277	256	236	216	
	252	300	273	254	210	
	252	286	270	262	216	
	252	**286.6**	**261.8**	**243.4**	**212**	**Mean**
	10.2323	8.792042	12.31666	16.69731	7.348469	**SD**
Diabetic +ELF	250	266	233	217	210	
	253	280	256	247	234	
	233	275	291	269	246	
	245	281	264	260	253	
	238	252	265	254	250	
	243.8	**270.8**	**261.8**	**249.4**	**238.6**	**Mean**
	8.288546	12.07063	20.80144	19.83179	17.54423	**SD**
Diabetic +AFF	230	258	233	218	195	
	248	275	250	226	210	
	239	260	246	217	187	
	253	275	245	224	208	
	257	280	267	231	198	
	245.4	**269.6**	**248.2**	**223.2**	**199.6**	**Mean**
	10.92245	9.914636	12.27599	5.80517	9.502631	**SD**
Diabetic +EFF	263	278	253	286	245	
	281	309	314	243	234	
	245	294	292	300	294	
	267	275	293	311	272	
	251	280	298	290	256	
	261.4	**287.2**	**290**	**286**	**260.2**	**Mean**
	14.09965	14.20211	22.48333	25.91332	23.54145	SD
Diabetic +ASF	268	304	290	284	260	
	236	267	270	267	254	
	240	264	293	288	260	
	254	272	282	280	252	
	237	256	273	271	246	
	247	**272.6**	**281.6**	**278**	**254.4**	**Mean**

	13.78405	18.48783	10.11435	8.803408	5.899152	SD
ESF	210	288	287	-	-	
	243	297	315	306	-	
	239	264	300	-	-	
	260	288	300	289	-	
	254	279	298	276	-	
	241.2	**283.2**	**300**	**290.3333**	**-**	**Mean**
	19.35717	12.47798	9.974969	15.04438	-	SD
Diabetic+ Metformin	252	257	240	210	169	
	248	259	231	217	196	
	231	242	225	202	185	
	250	261	253	214	174	
	233	242	230	212	189	
	242.8	**252.2**	**235.8**	**211**	**182.6**	**Mean**
	9.984989	9.418068	11.03177	5.656854	11.01363	SD
DC	255	273	294	322	347	
	245	279	315	317	328	
	254	299	311	327	344	
	238	282	290	304	320	
	242	280	297	318	344	
	246.8	**282.6**	**301.4**	**317.6**	**336.6**	**Mean**
	7.463243	9.762172	10.96814	8.561542	11.90798	SD
NC	67	62	90	85	100	
	70	74	93	107	99	
	69	90	79	88	93	
	67	85	111	94	96	
	65	75	95	98	95	
	67.6	**77.2**	**93.6**	**94.4**	**96.6**	**Mean**
	1.949359	10.84896	11.52389	8.677557	2.880972	SD

Appendix XI.Values for Animals' Body Weight Following Administration of Extracts -Fractions of Plant Parts

Period (Days)	Diabetic + ALF	Diabetic + ELF	Diabetic + AFF	Diabetic +EFF	Diabetic + ASF	Diabetic + metformin	Diabetic control	Normal control
Day 0	200	200	200	199	220	200	200	200
	205	204	198	202	201	210	205	200
	200	198	206	206	206	200	201	200
	210	213	202	197	198	207	200	198
	205	195	200	200	200	205	199	210
	180	160	180	146	187	178	170	205
Day 7	170	150	170	160	170	199	160	211
	176	163	160	157	180	167	175	217
	180	175	185	162	176	180	160	210
	194	170	176	178	180	175	170	200
Day 14	150	149	186	155	170	160	160	223
	150	140	170	170	162	180	150	210
	160	155	174	160	160	160	170	226
	173	160	194	174	158	170	148	217
	170	146	180	180	164	160	155	200
Day 21	165	170	186	168	170	173	155	227
	180	160	179	182	148	196	143	220
	170	160	195	168	150	180	164	230
	186	175	200	176	150	189	145	225
	188	160	197	187	157	173	140	210
Day 28	174	173	189	170	176	188	146	230
	199	169	181	186	150	200	143	228
	181	168	195	173	158	197	150	235
	193	180	207	185	161	200	140	230
	197	173	205	183	169	195	133	225

Appendix XII. Values for Fasting Blood Glucose of Animals Treate with Sub-Fractions of Ethanol-Aqueous Fraction of *Balanites aegyptiaca* leaves

GROUPS	Day 0	Day 7	Day 14	Day 21	Day 28	
Diabetic+L1	220	235	242	225	215	
	213	230	237	220	213	
	200	223	230	233	215	
	210	231	252	240	238	
	224	240	254	233	227	
	213.4	**231.8**	**243**	**230.2**	**221.6**	**Mean**
	9.316652	6.300794	10.0995	7.79102	10.71448	SD
Diabetic+L2	214	237	272	250	231	
	223	240	283	230	220	
	215	233	228	220	211	
	227	247	240	225	204	
	230	250	246	235	231	
	221.8	**241.4**	**253.8**	**232**	**219.4**	**Mean**
	7.120393	7.021396	22.91724	11.51086	12.01249	SD
Diabetic+L3	227	243	260	234	220	
	218	227	240	225	200	
	250	266	250	237	223	
	233	246	231	222	207	
	245	257	254	235	222	
	234.6	**247.8**	**247**	**230.6**	**214.4**	**Mean**
	13.0499	14.78851	11.53256	6.655825	10.31019	SD
Diabetic+L4	200	237	240	223	175	
	234	256	236	212	196	

	250	262	233	210	190	
	248	250	212	208	184	
.	242	270	244	217	200	
	234.8	**255**	**233**	**214**	**189**	**Mean**
	20.42547	12.49	12.4499	6.041523	9.899495	SD
Diabetic + Met	232	245	223	200	170	
	228	256	241	222	187	
	240	260	235	200	173	
	210	260	243	211	184	
	226	254	227	202	159	
	227.2	**255**	**233.8**	**207**	**174.6**	**Mean**
	11.00909	6.164414	8.671793	9.539392	11.28273	**SD**
Diabetic Cont.	215	233	247	265	300	
	205	229	252	300	318	
	224	249	269	312	324	
	218	232	258	298	310	
	212	230	245	278	304	
	214.8	**234.6**	**254.2**	**290.6**	**311.2**	**Mean**
	7.049823	8.203658	9.679876	18.80957	9.859006	SD
NC	70	68	80	79	86	
	56	60	73	111	110	
	68	80	79	89	93	
	72	78	90	100	96	
	65	70	86	80	84	

66.2	**71.2**	**81.6**	**91.8**	**93.8**	Mean
6.26099	8.074652	6.580274	13.66382	10.30534	SD

Appendix XIII. Values for Insulin Levels (pg/ml) of Animals Treated Sub-Fractions of Ethanol-Aqueous Fraction of *Balanites aegyptiaca*leaves

	Diabetic +L1	Diabetic +L2	Diabetic +L3	Diabetic +L4	Diabetic +Metformin	Diabetic Control	Normal Control
	76.21	72.58	102.82	75.81	79.03	57.66	58.47
	99.60	67.74	80.65	85.08	93.55	75.00	56.05
	71.77	75.40	75.40	88.31	100.4	71.77	101.61
	68.55	80.65	75.40	85.48	83.06	63.71	60.08
	63.71	82.66	77.02	81.05	65.72	63.31	77.07
Mean	**75.968**	**75.806**	**82.258**	**83.146**	**84.352**	**66.29**	**70.656**
SD	13.97711	6.043052	11.69276	4.849354	13.40975	6.998361	19.18968

Appendix XIV. Values for Biochemical Parametersof Animals Treated with Sub-Fractions of Ethanol-Aqueous Fraction of *Balanites aegyptiaca* leaves

	Glycogen (g/g liver)	Protein (mg/g liver)	Albumin (g/dl)	Fructosamine (mmol/L)
Diabetic +L1	15.25	15.53	2.59	0.38
	14.00	16.32	2.51	0.35
	14.25	16.84	3.09	0.40
	11.50	16.58	3.08	0.37
	12.75	16.97	2.54	0.36
Diabetic +L2	11.25	15.26	2.22	0.44
	11.75	15.66	2.59	0.41
	13.25	15.53	3.23	0.38
	12.50	15.79	3.22	0.40
	12.25	16.18	3.25	0.39

Diabetic +L3	15.25	16.84	3.16	0.33
	16.75	15.92	3.19	0.32
	15.50	17.11	3.27	0.34
	14.50	16.45	3.74	0.32
	11.75	17.24	3.14	0.35
Diabetic +L4	20.75	17.37	3.75	0.28
	16.75	18.55	3.69	0.25
	19.00	19.08	3.67	0.24
	22.00	20.00	3.58	0.27
	21.00	17.76	3.70	0.23
Diabetic Cont.	10.50	19.61	2.31	0.48
	11.75	19.08	2.29	0.52
	9.75	19.21	2.23	0.54
	13.00	19.47	1.81	0.49
	11.00	19.87	2.07	0.44
Diabetic + Metformin	21.75	22.76	3.83	0.18
	21.50	21.97	4.32	0.21
	21.00	21.71	4.12	0.29
	22.00	20.26	4.39	0.18
	23.75	18.42	4.37	0.22
Normal Cont.	25.00	23.42	5.02	0.15
	24.50	23.03	5.00	0.16
	21.75	24.47	4.95	0.20
	25.50	23.16	5.63	0.23
	23.00	23.68	5.00	0.25

Appendix XV. Values for Lipids profile of Animals Treated with Sub-Fractions of Ethanol-Aqueous Fraction of *Balanites aegyptiaca* leaves

GROUPS	TG (mg/dl)	Chol (mg/dl)	HDL- (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Diabetic +L1	187.85	174.14	55.17	125.53	37.57
	200.93	193.10	49.75	142.97	40.19
	188.79	186.21	53.69	137.71	37.76
	201.87	205.17	56.16	153.57	40.37
	206.54	194.83	59.11	141.70	41.31
Diabetic +L2	215.89	191.38	50.25	138.15	43.18
	205.61	203.45	49.75	152.38	41.12
	157.01	196.55	49.26	155.30	31.40
	219.63	193.10	50.74	139.03	43.93
	200.93	201.72	52.71	151.00	40.19
Diabetic +L3	217.76	182.76	58.13	127.58	43.55
	132.71	153.45	56.65	115.58	26.54
	114.95	156.90	54.68	122.97	22.99
	128.97	170.69	56.16	133.66	25.79
	120.56	146.55	54.19	111.60	24.11
Diabetic +L4	101.87	131.03	62.56	98.15	20.37
	116.82	98.28	69.46	61.02	23.36
	135.51	108.62	70.44	67.43	27.10
	117.76	101.72	67.98	64.58	23.55
	119.63	110.34	74.38	71.54	23.93
Diabetic Control	281.31	213.79	49.75	147.58	56.26

	267.29	198.28	48.28	135.16	53.46
	280.37	208.62	41.38	144.27	56.07
	271.03	203.45	36.95	141.85	54.21
	259.81	201.72	31.53	143.46	51.96
Diabetic + Metformin	95.33	82.76	70.94	49.51	19.07
	109.35	101.72	98.03	60.25	21.87
	117.76	118.97	74.38	80.54	23.55
	101.87	120.69	69.46	86.42	20.37
	93.46	106.90	68.97	74.41	18.69
Normal Cont.	73.83	86.21	87.68	53.90	14.77
	91.59	63.79	86.70	28.14	18.32
	92.52	82.76	71.43	49.97	18.50
	80.37	77.59	88.18	43.88	16.07
	65.42	96.55	89.16	65.64	13.08

Appendix XVI. Values forHepatic Glucose Metabolic Enzymes of Animals Treated with Sub-Fractions of Ethanol-Aqueous Fraction of*Balanites aegyptiaca*

	GK **(U/min/mg Protien)**	PFK **(U/min/mg Protien)**	PK **(U/min/mg Protien)**	G6Pase **(U/min/µmol Pi liberated)**	F16BPase **(U/min/µmol Pi liberated)**	PEPCK **(U/min/mg Protien)**
Diabetic +L1	2.42	4.47		1.45	2.13	0.21
	3.01	4.57	0.012765273	1.47	2.19	0.20
	3.07	4.39	0.012540193	1.52	2.27	0.21
	2.82	4.42		1.61	2.01	0.22
	2.53	4.61	0.013408360	1.64	2.17	0.21
Diabetic +L2	2.25	3.65	0.013344051	1.26	1.40	0.19

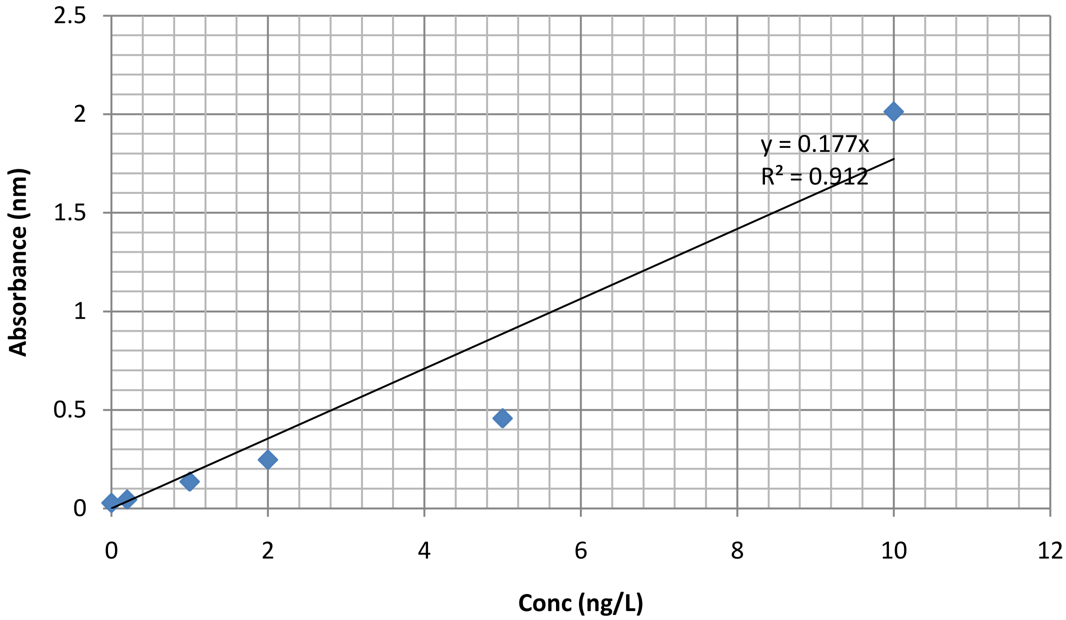
	2.89	3.59	0.012829582	1.37	1.65	0.19
	2.16	3.46	0.013311897	1.31	1.52	0.20
	3.18	3.63	0.012797428	1.47	1.51	0.20
	1.37	3.81	0.012861736	1.55	2.02	0.16
Diabetic +L3	2.77	5.00	0.012797428	0.83	1.55	0.18
	2.86	5.45	0.012958199	0.77	1.44	0.19
	2.82	5.61	0.015980707	0.43	1.37	0.18
	3.55	5.56	0.020932476	0.77	1.52	0.18
	3.07	5.59	0.019839228	0.60	1.53	0.17
Diabetic +L4	3.18	5.74	0.01903537	0.29	0.66	0.10
	3.41	5.47	0.018938907	0.34	0.79	0.10
	3.24	5.21	0.019646302	0.21	0.75	0.09
	3.28	5.42	0.022604502	0.28	0.87	0.10
	3.22	5.39	0.022315113	0.27	0.77	0.09
			0.021864952			
			0.022990354			

Diabetic Control	1.51	3.01	0.012475884	2.00	3.33	0.20
	1.45	3.20	0.009292605	1.83	3.53	0.19
	1.48	2.96	0.008938907	2.09	3.00	0.20
	1.51	3.17	0.008713826	1.89	2.84	0.20
	1.38	3.10	0.008585209	1.69	3.31	0.23
Diabetic + Metf.	5.32	6.01	0.024308682	0.20	0.65	0.02
	5.13	7.59	0.02392283	0.22	0.59	0.01
	5.24	6.17	0.023987138	0.25	0.61	0.01
	5.31	6.33	0.024662379	0.23	0.05	0.02
	5.25	6.19	0.02340836	0.29	0.01	0.02
Normal Cont.	5.60	7.01	0.023697749	0.12	0.59	0.007
	5.69	7.17	0.023601286	0.15	0.03	0.008
	5.59	6.99	0.023440514	0.19	0.63	0.010
	5.48	7.04	0.023054662	0.17	0.49	0.007
	5.68	7.17	0.023858521	0.14	0.28	0.013

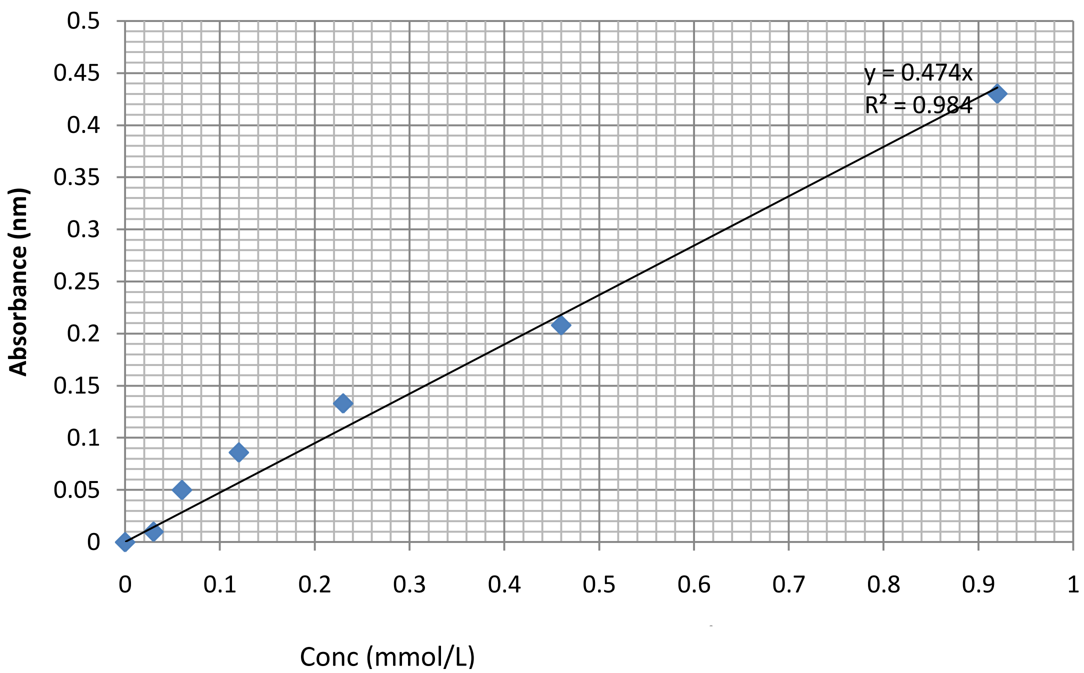
	GS**(U/min/mg Protien)**	GP**(U/min/mg Protien)**
Diabetic +L1	0.18	2.14
	0.18	2.10
	0.20	2.00
	0.20	1.56
	0.17	2.08
Diabetic +L2	0.10	2.29
	0.17	1.94
	0.15	1.47
	0.16	2.53
	0.17	2.65
Diabetic +L3	0.23	2.00
	0.23	1.94
	0.22	1.47
	0.23	1.56
	0.23	1.81
Diabetic +L4	0.24	0.80
	0.25	0.72
	0.25	1.01
	0.32	1.11
	0.33	1.47
Diabetic Control	0.10	2.89
	0.11	2.81
	0.09	2.45

	0.09	2.35
	0.09	2.30
Diabetic + Metf.	0.38	1.04
	0.37	0.95
	0.40	0.96
	0.37	0.98
	0.37	0.86
Normal Cont.	0.27	0.18
	0.38	0.33
	0.39	0.16
	0.33	0.17
	0.40	0.11

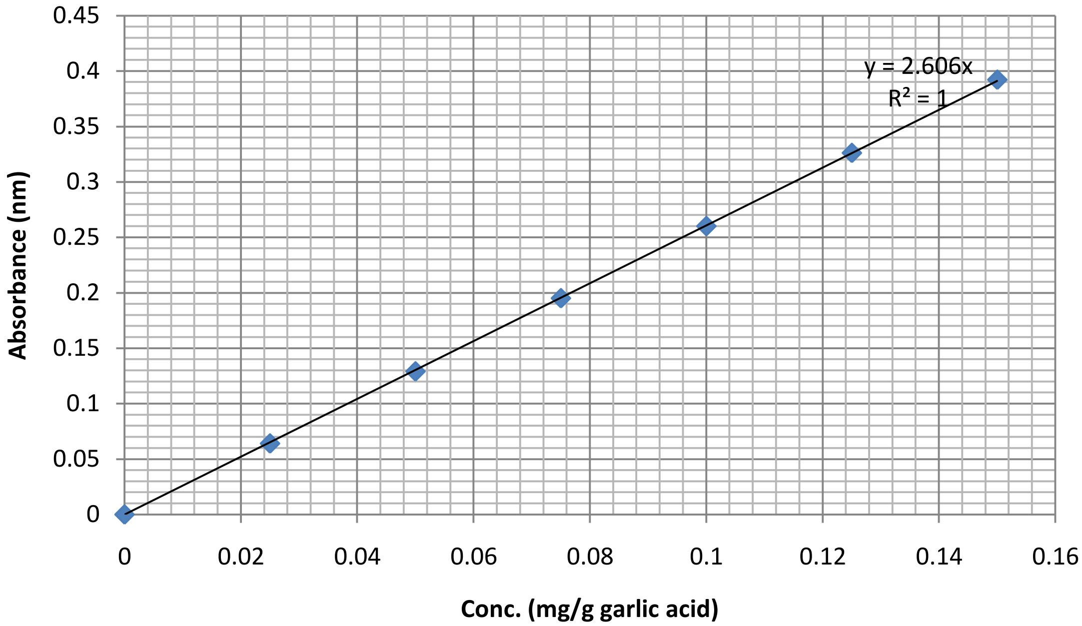
Appendix XVII.Calibration Curves for Standard Compounds



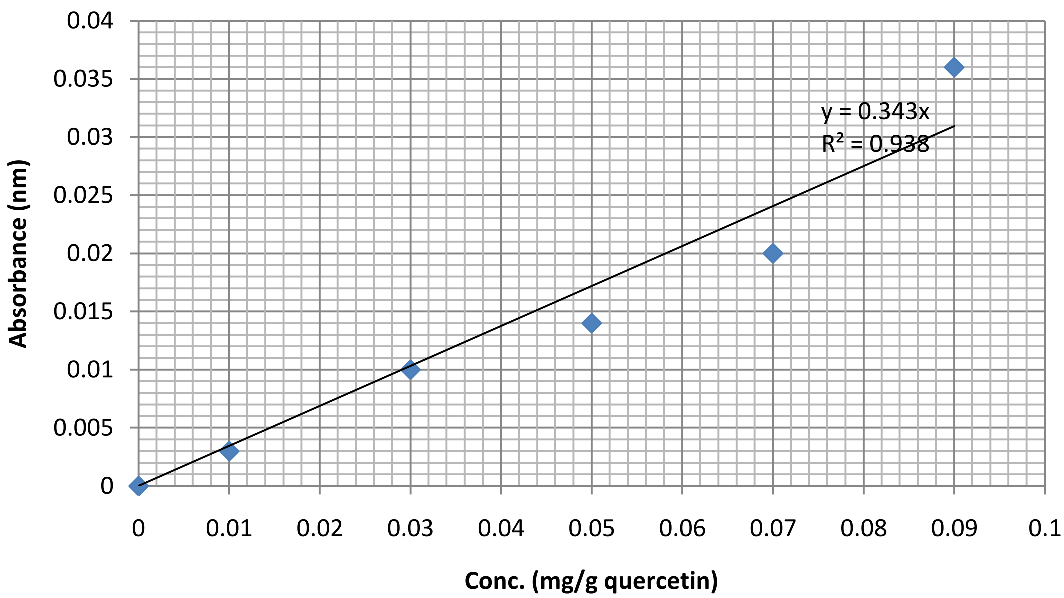
A. Calibrated insulin curve



B.Calibrated dihyroxylacetone curve



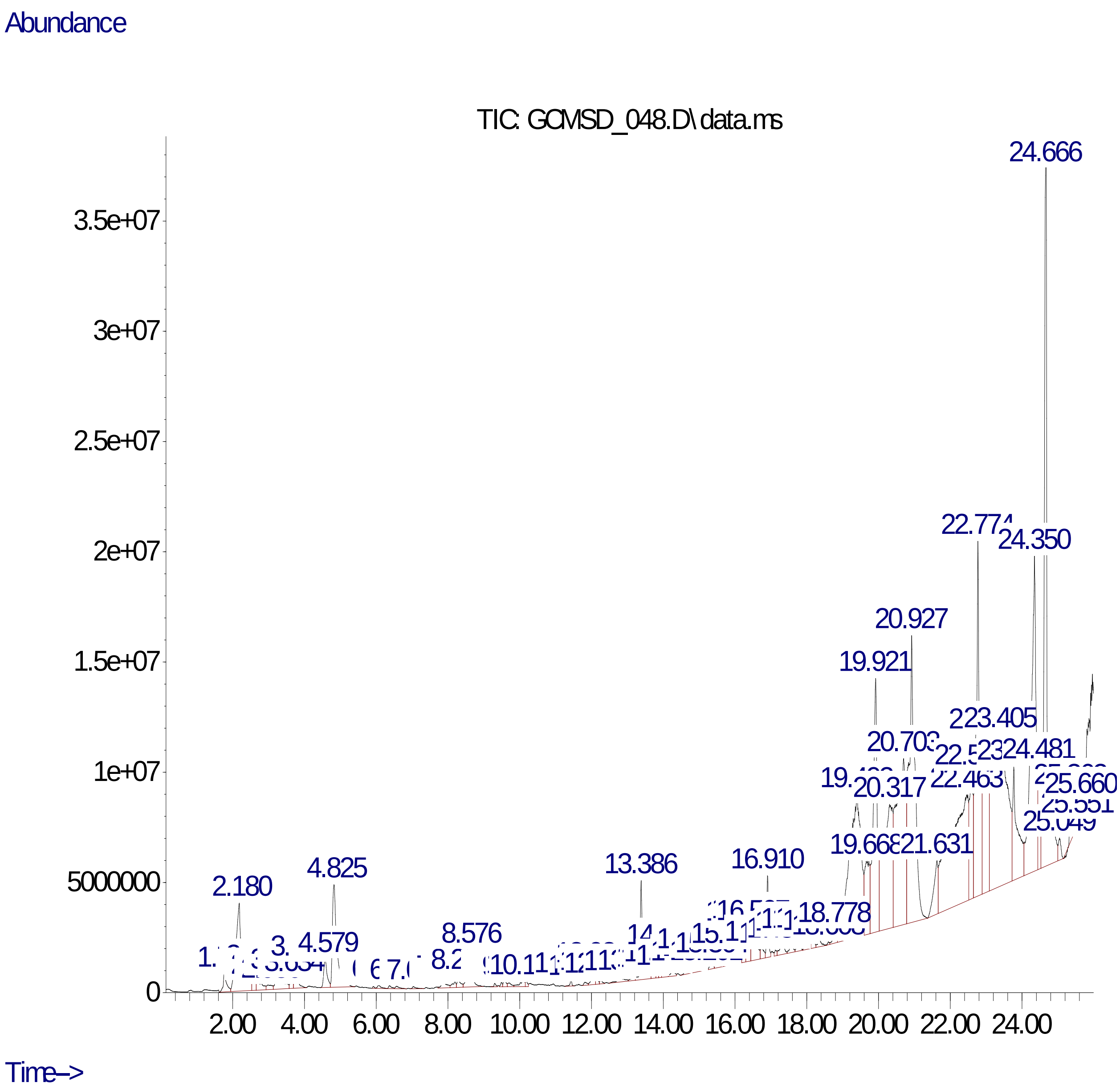
C. Calibrated phenolic (Garlic acid) curve



D. Calibrated flavonoids (quercetin) curve

Appendix XVIII. GC Chromatogram of Sub-Fractions (L4) Derived from Ethanol-

Aqueous Fraction of *Balanites aegyptiaca* Leaves



Appendix XIX: GCMS Analysis Results ofSub-Fractions (L4) Derived from Ethanol-

Aqueous Fraction of *Balanites aegyptiaca* Leaves

```
Data File : GCMSD_048.D (Extract-subfraction, L4)
Acq On    : 15 Dec 2016
```
INT TIC: GCMSD_L4.D\data.ms

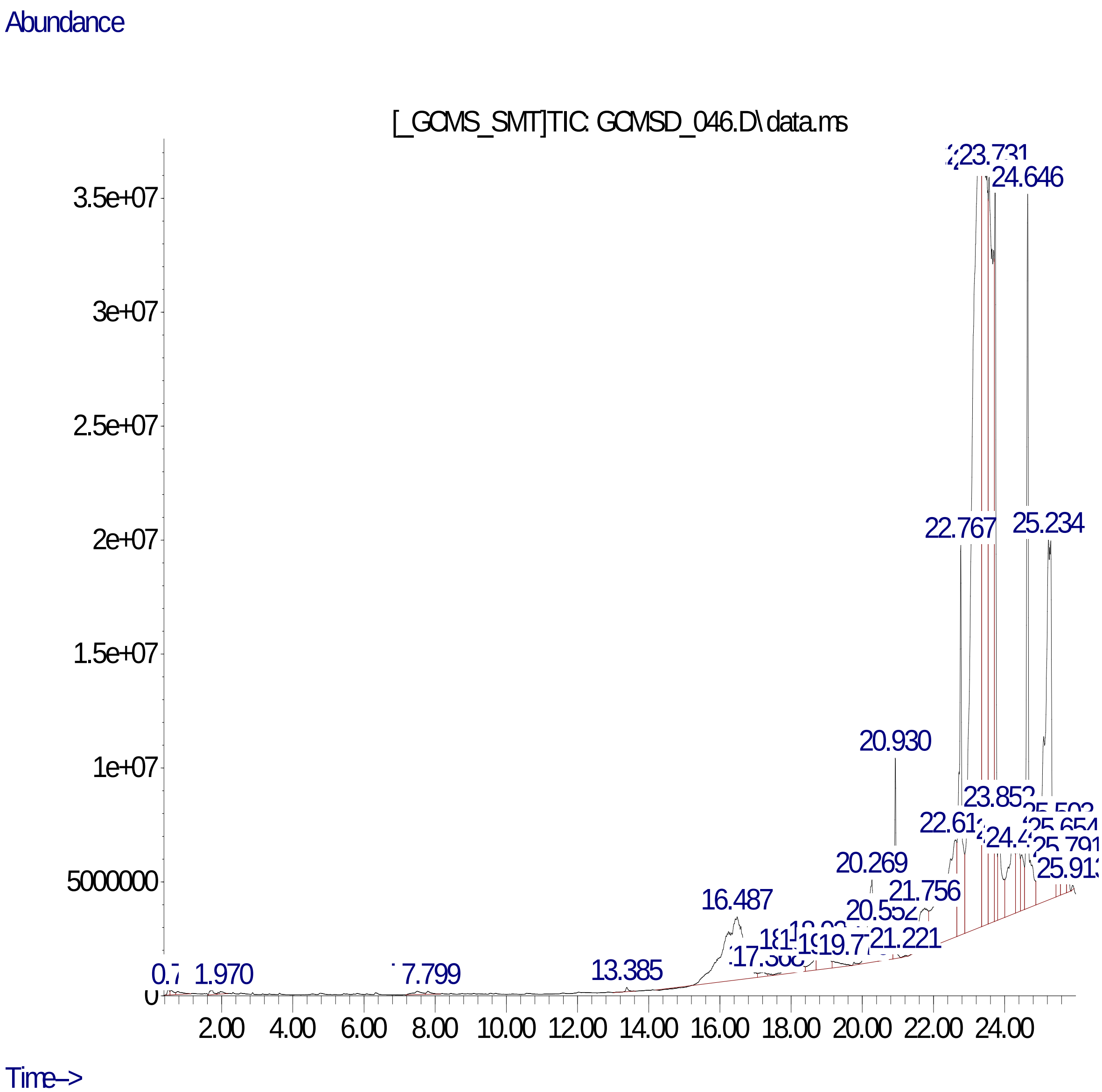
PK	RT	Area Pct	Library/ID	Ref	CASQUAL	
1	1.7683	0.2662	Propanal	222000123		9
2	2.18	2.205	Hydrazine, 1,1 dimethyl-	280	000057-5	
3	2.5714	0.18	(S)-(+)-Isoleucinol	8702	024629-25-2	12
4	2.7019	0.2225	Butanoic acid	2039	000107-92-6	37
5	2.9857	0.1077	Propanal, 3-methoxy-	2059	002806-84-0	47
6	3.2526	0.4257	Pyridine	1043	000110-86-1	83
7	3.6343	0.1087	Propanal, 3-methoxy-	2059	002806-84-0	22
8	3.8148	0.2795	1-Hexen-3-ol	3855	004798-44-1	12
9	4.5793	0.4047	1H-Pyrrole, 2,5-dimethyl-	2711	000625-84-3	90
10	4.8249	1.9724	1H-Pyrrole, 2,5-dimethyl-	2714	000625-84-3	80
11	5.9236	0.0132	1H-Pyrrole, 2,4-dimethyl-	2713	000625-82-1	38
12	6.0723	0.0494	Oxirane, 2-ethyl-2-methyl-	1819	030095-63-7	30
13	6.3743	0.0376	1H-Imidazole-2-methanol	3080	003724-26-3	46
14	6.5727	0.045	2,3,4-Trimethylpyrrole	5702	003855-78-5	53
15	7.0404	0.0331	Pyridine, 3-ethyl-	5261	000536-78-7	60
16	7.869	0.1313	N-Methylpivalamide	7939	006830-83-7	32
17	8.1951	0.088	N-(2-Cyanoethyl)-pyrrole	9516	043036-06-2	53
18	8.2833	0.1356	(S)-5-Hydroxymethyl-2[5H]-furanone	7266	078508-96-0	58
19	8.5757	0.5275	Benzyl alcohol	5466	000100-51-6	97
20	9.3061	0.0271	3-Hexene, 3,4-dimethyl-	6831	030951-95-2	38
21	9.4942	0.03	2-Methyl-2-butenenitrile	1140	004403-61-6	43
22	9.5705	0.0925	Mequinol	10609	000150-76-5	95
23	9.707	0.0617	Ethanone, 1-(1-cyclohexen-1-yl)-	10750	000932-66-1	22
24	10.0899	0.1288	4,5-Diamino-6-hydroxypyrimidine	11203	001672-50-0	35

25	10.1835	0.0456	4(1H)-Pyrimidinone, 5-hydroxy-2-methyl-	11275	024614-14-0	30
26	11.433	0.0471	Methyl salicylate	26605	000119-36-8	95
27	11.8237	0.0407	3-Methylpenta-1,3-diene-5-ol, (E)-	3326	001572-08-3	46
28	12.0319	0.1984	Benzofuran, 2,3-dihydro-	9568	000496-16-2	58
29	12.1407	0.0686	Phenol, 2-nitro-	18127	000088-75-5	60
30	12.2653	0.0421	4-Octyne	6000	001942-45-6	46
31	12.8141	0.1148	Phenol, 4-ethyl-2-methoxy-	26785	002785-89-9	83
32	13.192	0.0544	5H-1-Pyrindine	8736	000270-91-7	35
33	13.3861	1.0071	2-Methoxy-4-vinylphenol	25129	007786-61-0	95
34	13.7223	0.0663	Decanoyl chloride	55714	000112-13-0	27
35	13.8305	0.0305	6-Octadecenoic acid	142075	1000336-66-8	56
36	13.9305	0.0647	Phenol, 2,6-dimethoxy-	28298	000091-10-1	50
37	14.0089	0.2263	Eugenol	34529	000097-53-0	98
38	14.2719	0.0802	Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	53
39	14.6772	0.122	1-Hexadecanol	104433	036653-82-4	64
40	14.8513	0.1771	1,4-Benzenediol, 2-methoxy-	18799	000824-46-4	49
41	15.201	0.0471	3-Eicosene, (E)-	140277	074685-33-9	42
42	15.3636	0.0944	trans-Isoeugenol	34538	005932-68-3	95
43	15.807	0.4661	9-Octadecenal, (Z)-	126829	002423-10-1	95
44	16.1599	1.048	9-Octadecenal, (Z)-	126829	002423-10-1	95
45	16.2134	0.4701	9-Octadecenal, (Z)-	126829	002423-10-1	92
46	16.3752	0.5672	3-Eicosene, (E)-	140277	074685-33-9	91
47	16.507	0.6824	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	47508	002503-46-0	42
48	16.7413	0.1576	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	90
49	16.91	0.6004	4-Methyl-2,5-dimethoxybenzaldehyde	47437	004925-88-6	80
50	17.1342	0.0497	Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	97
51	17.3544	0.2046	2-Methyl-Z,Z-3,13-octadecadienol	140253	100090-5	92

52	17.5855	0.1626	Oleic Acid	142071	000112-80-1	56
53	17.7536	0.1661	Decanoic acid, 3-methyl-	52745	060308-82-9	25
54	18.0586	0.2148	Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	86
55	18.1642	0.1548	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	93
56	18.3542	0.1296	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	91
57	18.6082	0.022	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	92
58	18.7781	0.2025	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90-5	55
59	19.4019	7.3902	Glycerol 1-palmitate	188243	44-9	76
60	19.668	1.5631	Cyclohexene, 3R-acetamido-4cis,6cis-bis(acetoxy)-5trans-dimethylamino-	157039	1000153-71-4	35
61	19.9213	4.3763	Acetamide, N-methyl-N-[4-[3-fluoro-1-pyrrolidyl]-2-butynyl]-	75944	1000117-63-0	45
62	20.3173	5.1971	4,6-Di-O-methyl-.alpha.-d-galactose	72942	024462-98-4	47
63	20.7025	6.7886	Methyl 4-O-methyl-d-arabinopyranoside	46478	1000129-79-9	53
64	20.927	7.2708	Pentadecanoic acid, 14-methyl-, methyl ester	130843	005129-60-2	86
65	21.6306	0.9237	n-Hexadecanoic acid	117419	000057-10-3	93
66	22.4631	8.6743	n-Hexadecanoic acid	117419	000057-10-3	95
67	22.5898	1.8751	n-Hexadecanoic acid	117419	000057-10-3	90
68	22.7737	5.5695	11-Octadecenoic acid, methyl ester	155737	052380-33-3	99
69	22.9906	3.6057	n-Hexadecanoic acid	117419	000057-10-3	95
70	23.4051	10.5075	n-Hexadecanoic acid	117419	000057-10-3	93
71	23.7726	2.3987	Oleic Acid	142071	000112-80-1	93
72	24.35	6.8499	cis-9-Hexadecenal	100560	056219-04-6	98
73	24.4808	1.074	cis-9-Hexadecenal	100560	056219-04-6	98
74	24.6659	9.8185	Glycidyl palmitate	171251	1000383-37-8	95
75	25.0493	0.2538	Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	96
76	25.3619	0.4134	cis-13-Octadecenoic acid	142083	013126	95
77	25.5511	0.0149	cis-11-Hexadecenal	100562	053939-	95
78	25.66	0.0359	Cyclopentadecanone, 2-hydroxy-	102369	004727	95

Appendix XX.GC Chromatogram of Sub-Fractions (L4) Derived from Ethanol-

Aqueous Fraction of *Balanites aegyptiaca* Fruit-Mesocarp



Appendix XXI: GCMS Analysis Resultsof Sub-Fractions (L4) Derived from Ethanol-

Aqueous Fraction of *Balanites aegyptiaca* Fruit-Mesocarp

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Data File : GCMSD_046.D (methanol subfraction)
  Acq On    : 15 Dec 2016
```

INT TIC: GCMSD_F.D\data.ms

```
Pk#     RT   Area%          Library/NIST14.L           Ref#     CAS#   Qual
  1   0.501  0.05   5-Methyl-Z-5-docosene        180809 1000131-17-1 46
11,14-Eicosadienoic acid, methyl e 180765 002463-02-7 38
                 ster
1-Pentadecanol acetate             130789 000629-58-3 38

  2   0.590  0.06Dimethyl(2-octyl)amine            30155 007378-97-4 38
3-Decanone, 10-methoxy             52737 1000130-73-5 38
3-{4-[2-(Cyclopropylmethoxy)ethyl] 226581 1000408-10-0 38
                 phenoxy}-N-(1-methylethyl)-2-[(tri
                 methylsilyl)oxy]propan-1-amine

  3   0.769  0.06Butanoic acid, 3-methyl-            4361 000503-74-2 38
2H-1,2-Oxazine, 6-(4-chlorophenyl)  75172 015769-91-2 28
tetrahydro-2-methyl-
2-Thiophenecarboxylic acid, 5-(1,1  65597 054889-42-8 14
-dimethylethoxy)-

  4   1.731  0.07Benzeneacetonitrile, alpha.-[2-(d 138479 000125-79-1 38
imethylamino)propyl]-.alpha.-pheny
l-1,3-Propanediamine, N-(1-methyleth   8432 003360-16-5 27
yl)-Betaine                            8629 000107-43-7 22

  5   1.970  0.04Pentanoic acid                     4316 000109-52-4 17
Hydrazine, 1,1-dimethyl-              278 000057-14-7  9
Benserazide                        117801 000322-35-0  9

  6   7.499  0.10dl-Threitol                         9879 006968-16-7  9
1-Octadecanamine, N-methyl-        143110 002439-55-6  9
4-Decene, 2,2-dimethyl-, (Z)-       38365 055499-03-1  9

  7   7.799  0.052-Nitro-tertiary butanol            9218 005447-98-3 38
Butanal, 2-methyl-                   1764 000096-17-3 17
1-Pentene, 4,4-dimethyl-             3407 000762-62-9  9

  8  13.385  0.04 D:\MassHunter\Library\NIST14.L
                 2-Methoxy-4-vinylphenol            25129 007786-61-0 91
                 3-Methoxyacetophenone              25122 000586-37-8 64
                 Ethanone, 1-(2-hydroxy-5-methylphe  25241 001450-72-2 53
                 nyl)-

  9  16.487  4.769,17-Octadecadienal, (Z)-        125003 056554-35-9 94
                 9-Octadecenal, (Z)-               126829 002423-10-1 93
                 13-Octadecenal, (Z)-              126830 058594-45-9 91

 10  17.232  0.12 9,12-Octadecadienoic acid (Z,Z)- 140138 000060-33-3 95
                 Linoelaidic acid                  140124 000506-21-8 94
                 9,17-Octadecadienal, (Z)-         125003 056554-35-9 94
```

11	17.368	0.02	Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	97
			Linoelaidic acid	140124	000506-21-8	93
			9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	93
12	18.106	0.70	2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90	90
			9-Octadecenoic acid, (E)-	142089	000112-79-8	70
			13-Octadecenal, (Z)-	126830	058594-45-9	60
13	18.668	0.25	Oleic Acid	142071	000112-80-1	53
			7-Hexadecenoic acid, methyl ester, (Z)-	128697	056875-67-3	45
			Methacrylic acid, pentadecyl	155735	1000340-29-1	44
14	18.932	0.71	2-O-Methyl-D-mannopyranosa	60069	036864-61-6	43
			.alpha.-Methyl 4-O-methyl-D-mannoside	72944	1000127-87	43
			Cyclohexene, 3R-acetamido-4cis,6cis-bis(acetoxy)-5trans-dimethylamino-	157039	1000153-71	42
15	19.187	0.20	9,12-Octadecadienoic acid (Z,Z)-	140137	000060-33	56
			Oleic Acid	142071	000112-80-1	55
			Methacrylic acid, hexadecyl ester	169325	1000340-29	50
16	19.770	0.03	Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	96
			Z,E-2,13-Octadecadien-1-ol	126837	1000131-10-3	92
			Oleic Acid	142070	000112-80-1	91
17	20.269	1.89	Cyclooctanemethanol, .alpha.,.alph	39935	016624-06-9	38
			a.-dimethyl-Dodecanamide	64236	001120-16-7	38
			2-Octanol, 2,6-dimethyl-	30677	018479-57-7	35
18	20.552	0.92	Bis(2-ethylhexyl) phthalate	233372	000117-81	80
			Glutaric acid, 3-nitrophenethyl propyl ester	181107	1000376-74	35
			Benzyl butyl phthalate	171134	000085-68-7	35
19	20.930	0.93	Hexadecanoic acid, methyl ester	130813	000112-39-0	99
			Hexadecanoic acid, methyl ester	130820	000112-39-0	99
			Hexadecanoic acid, methyl ester	130822	000112-39-0	98
20	21.221	0.01	D:\MassHunter\Library\NIST14.L			
			Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	95
			Cyclopropaneoctanal, 2-octyl-	140252	056196-06-6	92
			13-Octadecenal, (Z)-	126830	058594-45-9	83
21	21.756	1.50	9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	93
			9,17-Octadecadienal, (Z)-	125003	056554-35-9	93
			cis-Vaccenic acid	142073	000506-17-2	92
22	22.619	4.96	trans-13-Octadecenoic acid	142094	000693-71-0	90
			Heptadecanolide	128637	005637-97-8	78
			9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	210570	003443-84-3	64
23	22.767	3.80	11-Octadecenoic acid, methyl ester	155737	052380-33-3	99
			cis-13-Octadecenoic acid, methyl e	155747	1000333-58	99

			ster			
			9-Octadecenoic acid, methyl ester, (E)-	155758	001937-62-8	99
24	23.330	25.63	10-Nonadecanone	142208	000504-57-4	15
			Butanal, 3-methyl-, oxime	4163	000626-90-4	14
			2-Methyl-tridecane-2,12-diol	93310	1000187-03-5	14
25	23.384	15.31	10-Nonadecanone	142208	000504-57-4	15
			.alpha.-D-Xylo-Hex-5-enofuranose, 5,6-dideoxy-1,2-O-(1-methylethylidene)-	53540	007284-07-3	14
			Succinic acid, tridec-2-methoxyethyl ester	208661	1000390-75-1	14
26	23.562	13.12	Cyclooctanemethanol, .alpha.,.alpha.-dimethyl-	39935	016624-06-9	22
			2-Hexanol, 2,5-dimethyl-, (S)-	14069	003730-60-7	14
			Butanal, 3-methyl-, oxime	4163	000626-90-4	14
27	23.731	4.53	2-Octanol, 2,6-dimethyl-	30664	018479-57-7	22
			2-(3,4-Dibromo-4-methylcyclohexyl)propan-2-ol	170160	110202-13-6	22
			7-Octen-2-ol, 2,6-dimethyl-	29230	018479-58-8	22
28	23.852	1.32	Menthane, 3-(2-methyl-2-hydroxy-propyl)-	76544	1000160-06-0	38
			4-Ethyl-3-octanol	30637	019781-26-1	32
			Dimethylallyl(n-octyl)silane	76354	081272-81-3	27
29	24.204	1.76	9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	90
			6-Octadecenoic acid, (Z)-	142084	000593-39-5	90
			cis-Vaccenic acid	142073	000506-17-2	87
30	24.396	0.92	1-Tricosene	180802	018835-32-0	93
			9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	93
			2-Methyl-Z,Z-3,13-octadecadienol	140253	1000130-90	90
31	24.481	0.63	1-Tricosene	180802	018835-32-0	95
			Cyclodocosane, ethyl-	194002	1000151-22	93
			Oleic Acid	142070	000112-80-1	86
32	24.646	5.06	Glycidyl palmitate	171251	1000383-37	95
			Myristoyl chloride	107807	000112-64-1	38
			1H-Tetrazole-1-ethanol, 5-amino-	13048	015284-29-4	27
33	25.234	9.06	Ethyl N-(p-bromophenyl)formimidate	90626	1000100-09-9	64
			1H-isoindole-1,3(2H)-dione, 2,2'-(1,2-ethanediyl)bis-	178563	1000402-31	38
			Decyl sulfide	173455	000693-83-4	30
34	25.503	0.57	Decyl sulfide	173455	000693-83-4	42
			2-Trifluoromethylbenzoic acid, 2-pentadecyl ester	238723	1000282-03	35
			4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione	42656	125039-11-4	30

35	25.654	0.53	Decyl sulfide	173455	000693-83-4	50
			3-Methyl-2-(4-trifluoromethyl-benzoylamino)-pentanoic acid	162193	1000300-09	43
			4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione	42656	125039-11-4	43
36	25.791	0.20	9,17-Octadecadienal, (Z)-	125003	056554-35-9	93
			cis-Vaccenic acid	142073	000506-17-2	90
			Oleic Acid	142071	000112-80-1	89
37	25.913	0.08	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	124319	309735-29-3	91
			7-Pentadecyne	72729	022089-89-0	90
			Erucic acid	195585	000112-86-7	90