Journal of Complementary and Alternative Medical Research

6(1): 1-12, 2018; Article no.JOCAMR.41997
ISSN: 2456-6276

Antihyperglycemic Effect of *Balanites aegyptiaca* Leaves Extract-Fractions in Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author DHM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KMA and IAU managed the analyses of the study. Author JOA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2018/41997
Editor(s):
(1) Dr. Arun Singh, Professor, Community Medicine, Rohilkhand Medical College & Hospital, Bareilly International University, India.
Reviewers:
(1) Idakwoji Precious Adejoh, University of Nigeria, Nsukka, Nigeria.
(2) Mohsen Kerkeni, University of Monastir, Tunisia.
(3) Rajagopal Karuppusamy, USA.
(4) Dennis Amaechi, Veritas University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/25299

Original Research Article

Received 10th April 2018
Accepted 19th June 2018
Published 27th June 2018

ABSTRACT

Introduction: The antidiabetic potentials of *Balanites aegyptiaca* fruit and seed extracts has been reported by several scientific studies. While very few data reported anantidiabetic study of the plant leaf despite it's used by some traditional medicine practitioners in the management of diabetes mellitus and some ailments. This study, therefore, investigates the effect of *Balanites aegyptiaca* leaves extracts in streptozotocin-induced diabetic rats in a bid to ascertain its antidiabetic potential.
Methods: Dried powdered *Balanites aegyptiaca* leaves was defatted with hexane and then extracted with ethanol. The ethanol extract was a petition with water and ethyl acetate (1:1 v/v) and then separated. Ethyl acetate (ELF) and aqueous (ALF) fractions obtained were studied for antihyperglycemic activity. Diabetes mellitus was induced in male wistar rats by intra-peritoneal injection of streptozotocin (60 mg/kg body weight).

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Results: The results showed that diabetes-induced in rats was characterized by low serum insulin and hyperglycemia. Treating diabetic rats with extract-fractions of *Balanites aegyptiaca* leaves slightly elevated serum insulin, lowered fasting blood glucose levels and improved serum lipid profile; total cholesterol, TG, LDL-C and VLDL-C toward normal. The aqueous fraction (ALF) was the most potent; it lowered fasting blood glucose by 15. 87% compared to the 24. 62 % fasting blood glucose reduction by metformin. ALF of *Balanites aegyptiaca* leaves 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 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).

Conclusion: In conclusion, the results of the study showed that *Balanites aegyptiaca* leaves ethanol extract-fractions exerted antihyperglycemic, antilipidemic and glucose enzymes regulatory effects. Further research is needed to explore the leaves bioactive components and their mode of action so that the plant leaves with the bioactive compounds can be used as an active pharmaceutical ingredient for drug medication manufacturing.

Keywords: Balanite aegyptiaca; leaves; extract-fractions; antidiabetic; rats.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders. Is one of the key health problem, affecting millions of people worldwide with a high-frequency rate and is projected to reach 592 million people in the year 2035 [1]. Diabetes mellitus occurs due to either low plasma insulin level or insensitivity of target organs to insulin and is characterized by a chronic hyperglycemia. There are several types of diabetes, but the two most common types are type 1 and type 2 diabetes. Type 1 diabetes is caused by an auto-immune response leading to a breakdown of insulin-producing cells [2], while type 2 diabetes mellitus resulted from insulin resistance and beta-cell failure which resulted from a progressive insulin secretory defect on the background of insulin resistance [3].

The search for effective antidiabetic agents particularly from nature products such as medicinal plants for the management of Diabetes mellitus is highly recommended considering the prevalence and complication of, and death rate caused by the disease as well as the side effects of the available synthetic antidiabetic drugs. Literature surveyed shows that research on medicinal plants like *Galega officinalis*, has yielded positive results by identifying metformin as its active antidiabetic compound [4]. On this note, the emphasis of research has been on utilizing medicinal plants that have long and proven history of curing or treating various ailments [5].

The plant *'Balanites aegyptiaca Delile*', also known as 'desert date' in English, a member of *Zygophyllaceae* family, is a common plant species of the dry land areas of Africa and Asia [6,7]. 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 range of diseases [8].

Balanites aegyptiaca extracts have been reported to exert antihyperglycemic activity in experimental induced diabetic animals' mode [9, 10]. The fruit and seed extracts are the most widely studied and reported to have exhibited prominent antihyperglycemic activity and also improved lipid profile toward normal levels in diabetic-induced animals [11,12,13]. *Balanites aegyptiaca* fruit extract was reported to have stimulated insulin secretion [11,14], inhibited intestinal α-amylase activity [15], and increased muscle basal glucose uptake [10] to lowered blood glucose level while the seed extract was reported to have exerted antihyperglycemic effect by ameliorating beta-cell dysfunction [13] and antioxidant activity as suggested by Shafik et al. [16]. In a recent study, it was reported that the leaf extract stimulated erythrocytes glucose uptake in type II diabetic patients [17] while Gawade and Farooqui [18] reported that it inhibited alpha amylase activity *in vitro*.

Literature surveyed showed that there are very few experimental evidence that carried out antidiabetic study of *Balanites aegyptiaca* leaves despite the reports that the plant leaves is used by some traditional medicine practitioners in the management of diabetes mellitus and some ailments [8,19]. This study therefore aimed at

investigating effect of *Balanites aegyptiaca* leaves extract in streptozotocin-induced diabetic rats in a bid to ascertain antihyperglycemic potential.

2. MATERIALS AND METHODS

2.1 Chemicals/Reagents

All chemicals and reagents used were of analytical grade and obtained from Sigma Aldrich, USA and BDH Ltd Poole, England.

2.2 Experimental Plant Material

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

2.3 Extraction of Plant

Plant leaves was defatted as performed by Jung et al. [20] and extracted as done by Govorko et al. [21] with little modification in the choice of the extraction temperature (60°C). Seven hundred and fifty gram (750 g) powdered of plant leaves was defatted for 2 hours with 1200 ml hexane on a mechanical shaker. The hexane solvent was discarded, then the defatted sample air-dried. Exactly 200 g of the defatted plant leaves was mixed with 2000 ml of 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. The ethanol leaves extract was dissolved in water (500 ml) and partitioned with ethyl acetate (500 ml) at 20°C for 2 hours then separated using a separating funnel (1000 ml). Fractions were concentrated using a rotary evaporator at 40 ^{o}C and air dried. The dried aqueous (ALF) and ethyl acetate (ELF) fractions of *Balanites aegyptiaca* leaves were stored in air-tight containers and kept in a refrigerator at 4°C until used.

2.4 Experimental Animals

A total of twenty-five (25) male wistar albino rats were used for the study. The rats were obtained from the Animal House, University of Jos, Plateau State, Nigeria 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 grow attaining a weight between 180-230g before used. All experimental protocol was in conformity with the institutional guidelines that are in compliance with national and international laws and guidelines for care and use of laboratory animals [22].

2.5 Induction of Diabetes Mellitus

Diabetes mellitus was induced in rats by intra-peritoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body wt dissolved in 0.1 M citrate buffer (pH 4.5). Rats were given 10 % 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 subsequent 1-week intervals to identify the onset and continued presence of diabetic hyperglycemia; rats with fasting blood glucose levels ≥200 mg/dl were considered diabetic and selected for the study [23].

2.6 Experimental Design

Antihyperglycemic effects of ethanolic extract-fractions of *Balanites aegyptiaca* leaves were assessed in the streptozotocin-induced diabetic rats. Rats were randomly allocated into groups of 5 rats each as follows;

Group A : Diabetic + Aqueous leaf fraction (ALF)
Group B : Diabetic + Ethyl acetate leaf fraction (ELF)
Group C : Diabetic + Metformin at 200 mg/kg body weight (kolawole and Akanji [24].
Group E : Diabetic control
Group F : Normal control

At the end of the experiment, animals were sacrificed, blood was collected and serum separated was used for some biochemical parameters assessments. Hepatic key glucose metabolic enzymes like Glucokinase [25], Phosphofructokinase [26], Fructose-1,6-bisphosphatase [27], Phosphoenolpyruvate carboxylkinase [28], Glucose-6-phosphate dehydrogenase [29], Glycogen phosphorylase activity [30], Glucose-6-phosphatase activity [31], Glycogen synthase activity [32], Pyruvate kinase (PK) [33] were assayed.

The extract-fractions were administered orally using oral gastric tube. Exactly, 400 mg/kg body weight of plant extracts were administered to various diabetic rats' groups for 28 days period. The extract-dose used was determined following our previous acute toxicity report on the ALF and ELF of *Balanites aegyptiaca* [34].

2.7 Determination of Blood Glucose Levels

Blood glucose levels were determined by method described by Beach and Turner, [35]. Principle: Glucose oxidase catalyses the oxidation of glucose to form glucuronic acid and H_2O_2. H_2O_2 reacts with 4–aminoantipyrine and 4-chlorophenol in the presence of peroxidase to produce red chromogen.

2.8 Determination of Serum Insulin Levels

Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) method as described by Clark and Hales [36]. 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.

2.9 Determinations of Lipid Profile Levels

Determination of Serum triglyceride (TG) was done by the method of Fossati and Prenape [37], Total Cholesterol [38], High Density Lipoprotein Cholesterol (HDL-C) [39] while LDL Cholesterol and VLD L Cholesterol (VLDL-C) were estimated using the formula described by Marchell [40]: LDL – Cholesterol conc. (mg/dL) = [TC - (HDL-C + Triglycerides /5)]and VLDL-Cholesterol conc. (mg/dL) = [Triglycerides /5]. All assay were done using assay kits from Agappe Diagnostics Switzerland GmbH.

2.10 Liver Function Test

Total Protein was determined by method described by Bradford [41]. This is based on the formation of blue colored complex when protein reacts with commassie dye under acidic condition. The protein is measured spectrophotometrically at 595 nm. The assay was done using assay kit from Agappe Diagnostics Switzerland GmbH. Serum Albumin was determined by method described by Doumax and Watson [42]. 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 578 nm.

2.11 Statistical 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) [43]. Significant difference was accepted at $P < 0.05$.

3. RESULTS

3.1 Antidiabetic Effect of ELF and ALF in STZ-Induced Diabetic Rats

The effect of *Balanites aegyptiaca* ethyl acetate leaves fraction (ELF) and aqueous leaves (ALF) fraction on blood glucose level in STZ diabetic rats is given in Fig. 1. In the diabetic untreated rats, levels of fasting blood glucose gradually raised and continued throughout the experimental period from 246.80±7.46 mg/dl to 336.69±11.91 mg/dl. A significant (*P<0.05*) decreased in fasting blood glucose levels in diabetic rats treated with the metformin and plant extract-fractions were recorded. Diabetic rats treated with metformin had a fall in fasting blood glucose by 24.62% followed by the group that received aqueous leaves fraction (ALF) 15.87 %. Aqueous leaves fraction of the plant was the most potent.

3.2 Effect of ELF and ALF on Insulin Level in Diabetic Rats

Increased in serum insulin levels were recorded following oral administration of ELF and ALF of *Balanites aegyptiaca* to diabetic rats (Fig. 2). Serum insulin levels of diabetic control rats was 0.09±0.02 ng/L which was significantly different (P<0.05) from diabetic rats groups treated with ALF (0.27±0.04 ng/L) and ELF (0.22±0.06 ng/L). However, the diabetic rats treated with ALF seem to have their serum insulin increased then ELF.

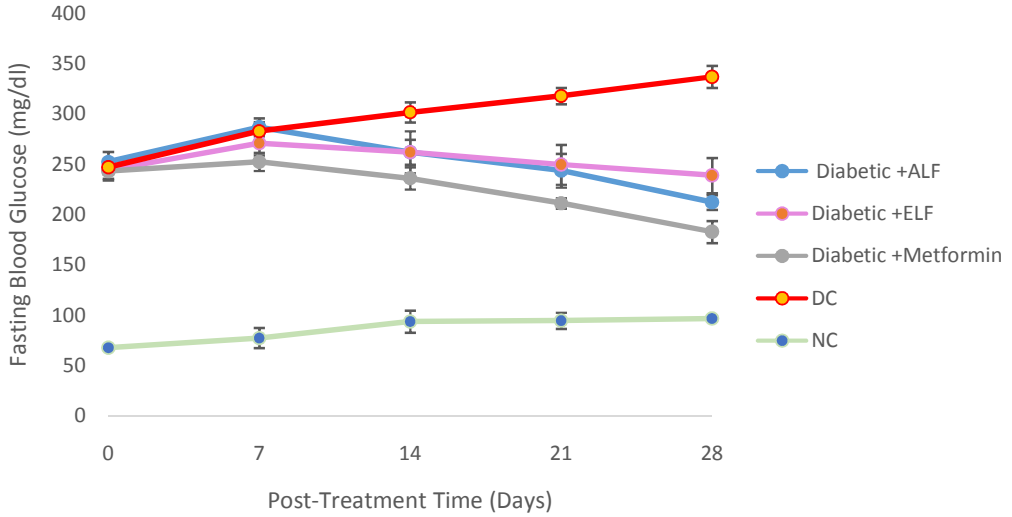

Fig. 1. Anti-hyperglycemic Effect of Ethanol-Extract Fractions of *Balanites aegyptiaca* Leaves in Streptozotocin-induced Diabetic Rats

ALF = Aqueous Leaves Fraction, ELF = Ethyl Acetate Leaves Fraction, DC = Diabetic Control, NC = Normal Control

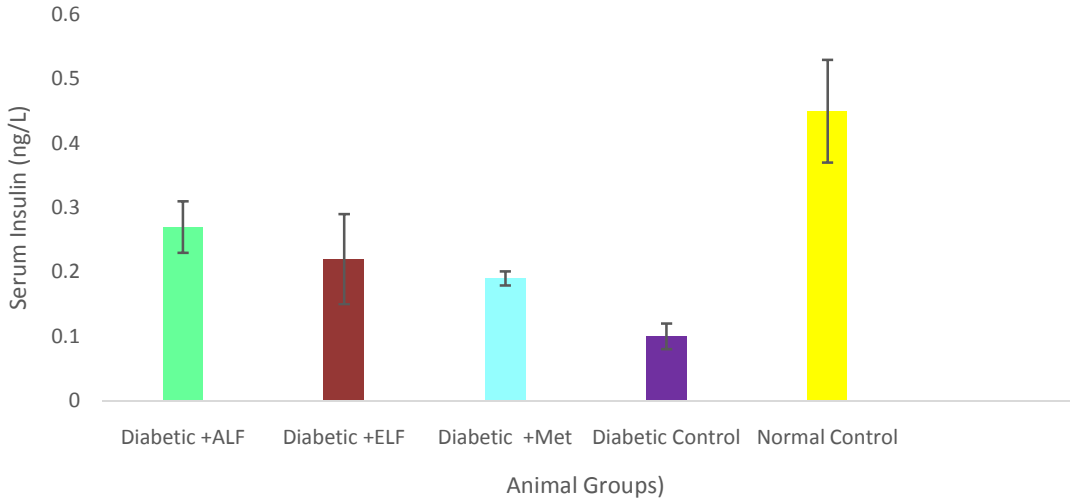

Fig. 2. Effect of Oral Administration of Ethanol Extract-Fractions/Subfractions of *Balanites aegyptiaca* Leaves on Serum Insulin Level of Streptozotocin-Induced Diabetic Rats

ALF = Aqueous Leaves Fraction, ELF = Ethyl Acetate Leaves Fraction

3.3 Effect of ELF and ALF on Biochemical Parameters in Diabetic Rats

STZ induced diabetic rats showed significant increase in cholesterol, TG, VLDL and decrease in HDL levels compared to normal and diabetic treated rats. While administration of ethyl acetate leaves fraction (ELF) and aqueous leaves fraction (ALF) of *Balanites aegyptiaca* to various diabetic rats groups significantly reserves lipid profile as shown in Table 1. Serum cholesterol level in diabetic untreated rats was 232.00±2.96 mg/dl which is significantly different ($P<0.05$) compared to diabetic rats treated with metformin (180.62±3.19 mg/dl) and the ALF (172.00±2.96 mg/dl). Similarly, elevated serum triglycerides levels were significantly ($P<0.05$) reduced in diabetic rats treated with ALF (119.91±2.60 mg/dl) whereas HDL-cholesterol was elevated in

same rats groups compared to diabetic untreated rats. Ethanol-aqueous fractions of the plant leaves was the most potent. There was significant (*P<0.05*) decrease in the concentration of albumin of untreated diabetic rats (2.21±0.16 g/dl) compared to all the diabetic treated groups.

3.4 Effect of ELF and ALF on Glucose Metabolic Enzymes in Diabetic Rats

From the diabetic rats groups that were treated with the ALF and ELF, there was a significant (*P < 0.05*) changes in the enzymes activites (Table 2). Activities of glycolytic enzymes namely; glucokinase (GK), phosphofructo kinase (PFK), and pyruvate kinase (PK) determined in liver tissues of STZ-induced diabetic rats. Activities of these key enzymes assayed in liver tissues of diabetic control rats were suppressed but enhanced in diabetic rats treated groups.

Change in glycogen synthase (GS) and phosphorylase in liver of diabetic treated rats were reversed compared to diabetic untreated rats. Activities of 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 also shown in Table 2. 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 to 0.14±0.01 U/min/µmole P_i liberated), fructose-1,6-bisphosphatase (from 2.19±0.25 to 1.32±0.06 U/min/µmole P_i liberated), phosphoenol pyruvate carboxylkinase (0.17±0.01 U/min/mg protein).

4. DISCUSSION

Different plants part have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Literature surveyed showed *Balanites aegyptiaca* fruit and seed extracts widely studied as antidiabetic agents where some bioactive compounds and their likely mode of action were profiled. While, very few experimental evidences had studied the antidiabetic effect of the plant leaf despite its used by some traditional medicine practitioners in the management of diabetes mellitus and some ailments [8,44] as well as the presence of some biologically active compounds in the leaves [45,46,47]. In this study, the antihyperglycemic effect of ethanol extract-fractions (ELF and ALF) of *Balanites aegyptiaca* leaves were investigated in streptozotocin induced diabetic rats. This was to ascertain antidiabetic efficacy of the plant leaves.

Decreased fasting blood glucose levels from the diabetic rats groups that received extract-fractions of *Balanites aegyptiaca* leaves implies that it possess antidiabetic property. From our previous study, we found that ethanol extract-fractions of *Balanites aegyptiaca* leaves have the ability to lowered blood glucose in glucose-loaded rats which were further substantiated by their improved glucose tolerance in diabetic-induced rats suggesting that this plant's leaves extract-fractions contain compounds that have the capacity to correct impaired glucose tolerance in diabetes mellitus, hence may exhibit antidiabetes [35]

Type 1 diabetes mellitus is characterized by severe loss of body weight that resulted from a relative or absolute deficiency of insulin due to defective β- cells [48]. A significant decrease in body weight of untreated STZ-induced diabetic rats observed may be attributed to low insulin levels in those rats. The loss of weight in STZ-induced diabetic rats might be accompanied by an increased breakdown of muscle proteins (for provision of gluconeogenic amino acids) in these rats [48], suggesting degradation of structural protein as contributing factor towards weight loss. Continuous treatments of STZ-induced diabetic rats with the fractions of ethanolic extract of *Balanite aegyptiaca* leaves significantly prevented body 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 [49]. 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 [50,51]. Insulin also increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes mellitus causes hypercho-lesterolemia [39] which may explain high serum cholesterol recorded from the untreated diabetic rats.

Table 1. Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves on Biochemical Parameters in Streptozotocin-Induced Diabetic Rats

	Animals Grouping				
	Diabetic + ALF	**Diabetic + ELF**	**DC**	**Diabetic + Metformin**	**NC**
Cholesterol (mg/dl)	172.00±2.96^{b}	224.61±13.10^{bcd}	232.00± 2.96^{bcde}	180.62±3.19^{bc}	76.31±3.19^{a}
Triglyceride (mg/dl)	119.91±2.60^{b}	185.39±5.73^{bcd}	207.13± 6.05^{bcde}	99.91±5.55^{a}	97.82±2.90^{a}
HDL-Cholesterol (mg/dl)	50.00±0.66^{b}	60.87±1.59^{bc}	27.22±5.07^{a}	66.35±5.53^{bcd}	47.78±3.01^{b}
LDL-Cholesterol (mg/dl)	138.02±3.03^{bc}	175.37± 12.98^{bcde}	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}	41.43±1.21^{bcde}	19.98±1.11^{a}	19.57±0.58^{a}
Fructosamine (mmol/L) x10^{-1}	0.83±0.19^{bc}	0.67±0.12^{b}	2.91±0.70^{bcd}	0.48±0.01^{b}	0.30±0.16^{a}
Albumin(g/dl)	4.00±0.01^{b}	2.44±0.01^{a}	2.21±0.16^{a}	3.59±0.00^{b}	4.28±0.01^{bc}
Total Protein(mg/g liver)	21.58±0.06^{b}	20.85±0.17^{b}	17.35±1.96^{a}	25.01±0.06^{bc}	26.95±0.04^{bcd}

Values are Mean ± SD of 5 determinants. Values with different superscript across the rows are significantly different (P<0.05)
ALF = Aqueous Leaves Fraction, ELF = Ethyl Acetate Leaves Fraction, DC = Diabetic control, NC = Normal Control

Table 2. Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Leaves on Glucose Metabolic Enzymes in STZ-Induced Diabetic Rats

	Animals Grouping				
	Diabetic + ALF	**Diabetic + ELF**	**DC**	**Diabetic + Metformin**	**NC**
Glucokinase (U/min/mg Protein)	2.75±0.01^{b}	2.23±0.00^{a}	2.22±0.02^{a}	2.72±0.02^{b}	3.53±0.01^{bc}
Phosphofructokinase (U/min/mg Protein)	2.52±0.03^{bc}	2.23±0.01^{b}	2.06±0.07^{a}	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}	0.04±0.01^{a}	0.15±0.02^{bc}	0.11±0.01^{b}
Glycogen Phosphorylase (U/min/mg Protein)	2.76±0.02^{b}	2.75±0.01^{b}	3.82±0.21^{bc}	2.04±0.01^{a}	2.07±0.01^{a}
Glycogen Synthase (U/min/mg Protein) x10^{-2}	14.45±0.16^{bc}	12.00±0.22^{b}	9.41±0.34^{a}	15.51±0.42^{bcd}	29.25±0.88^{bcde}
Glucose-6-Phosphatase (U/min/µmole P_i liberated)	0.14±0.01^{a}	0.15±0.01^{a}	1.44±0.05^{bc}	0.12±0.02^{a}	0.07±0.01^{a}
Fructose-1,6-BisPhosphatase (U/min/µmole P_i liberated)	1.32±0.06^{b}	1.43±0.17^{bc}	2.19±0.25^{bcd}	1.02±0.02^{a}	1.40±0.07^{bc}
Phosphoenol-pyruvate Carboxyl kinase (U/min/mg protein)	0.17±0.01^{ab}	0.21±0.01^{b}	0.81±0.15^{bc}	0.11±0.04^{a}	0.09±0.01^{a}

Values are Mean ± SD of 5 determinants. Values with different superscript across the rows are significantly different (P<0.05)
ALF = Aqueous Leaves Fraction, ELF = Ethyl acetate leaves Fraction

In this study, the high levels of cholesterol in diabetic rats observed were reversed in STZ induced diabetic rats treated with the fractions of ethanolic extract of *Balanites aegyptiaca* leaves significantly. 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 [52]. 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 extracts since study has shown an increased HMG-CoA reductase activity in diabetic rats [51].

It has been reported that, hyper-triglyceridemia that characterizes the diabetic state is a consequence of uninhibited actions of pancreatic lipase [53]. From this study, it may be assumed that administration of ethanol extract-fractions of *Balanites aegyptiaca* leaves 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 in those rats [54,55]. These findings agreed with the report by Samir et al. [11] that aqueous and ethanolic extracts of *Balanites aegyptiaca* fruit were able to decrease serum total cholesterol and triglycerides in STZ-induced diabetic rats.

Decrease in albumin may be due to albuminuria which are important clinical markers of diabetic nephropathy or glycation [56,57] and might also be due to increased protein catabolism [58]. Treating diabetic rats with the ethanol extract-fractions of *Balanites aegyptiaca* leaves has 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 [59].

Activities of glucokinase, phosphofructokinase and pyruvate kinase has been shown to be very sensitive signs of the glycolytic pathway and these are decreased in the liver of diabetic state [60]. Reduced activities of these enzymes in this study are consistent with other studies on glycolytic enzymes [61,62]. 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 [63]. Insulin influences the intracellular utilization of glucose by promoting glycolysis by increasing the activity and amount of glycolytic enzymes [61,64]. Administration of fractions of the ethanolic extract of *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 [65,66].

Some plants extract have been reported to regulated glycogen enzymes leading to increased hepatic glycogen content [67,68]. According to Gutierrez [69], activation of glycogen synthase by plant suggested insulinogenic character; going by this statement one may propose that *Balanites aegyptiaca* leaves contains component that exhibit insulin like effect since the altered activities of glycogen synthase and phosphorylase were reversed in diabetic rats treated with the extract-fractions of *Balanites aegyptiaca* leaves.

Gluconeogenesis is a main cause of the elevated hepatic glucose production contributing 50-60% of the released glucose [70]. Insulin decreases gluconeogenesis by decreasing the activities of enzymes, such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase [60, 71]. In this study extract-fractions of *Balanites aegyptiaca* leaves seem to have suppressed the activities of these enzymes as reported by several studies that plants extract were able to suppressed the activities gluconeogenic enzymes in diabetic animals [24,59,72]. Shafik et al. [16] have reported that extract of *Balanites aegyptiaca* seed-kernel suppressed glucose-6-phosphatase activity

Recent study has reported some compounds in the leaf extract of *Balanites aegyptiaca* [18]. The compounds 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. The hypoglycemic properties

of *Balanites aegyptiaca* has been attributed to the presence of some of the above mention compounds like phenolics and flavonoids. Study by Al-Malki et al. [73] has reported two phenolics; vanillic and syringic acids from fruit extract of the *Balanites aegyptiaca* while rutin and isorhamnetin was reported in the fruit extract by Motaal et al. [10]. In line with the above reports, *Balanies aegyptiaca* leaves extract may have regulated glucose enzymes to improved glycemic control due to some phenolic compounds contain by the leaves.

5. CONCLUSION

The study concluded that ethanol extract-fractions of *Balanites aegyptiaca* leaves exerted antihyperglycemic and antihyperlipidemic effects in STZ-induced diabetic rats. The study confirmed leaves extract of *Balanites aegyptiaca* has potential antidiabetic activity. Further research is needed to explore the leaves bioactive component and their mode of action so that the plant with the bioactive compounds can be used as active pharmaceutical ingredient for drug medication manufacturing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authors hereby declare that all 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 publication No. 85-23, revised 1985).

ACKNOWLEDGEMENT

We acknowledge the efforts of Mallam Adamu Mohammed and Mr. Kabir Abdullahi both from the Department of Pharmagonosy, ABU Zaria, Nigeria for their technical assistance during the extraction/fractionations of plant leaves and the TLC analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Research and Clinical Practice. 2014;103:137–149.
2. Delmastro MM, Piganelli JD. Oxidative stress and redox modulation potential in type 1 diabetes. Clin Dev Immunol. 2011; 1:9764-9774.
3. NDIC (National Diabetes Information Clearinghouse). National Diabetes Statistic. 2011; Available:www.diabetes.niddk.nih.gov
4. Andrade-Cetto A. Effects of medicinal plant extract on glyconeogenesis. Botanics: Targets and Therapy. 2012;2: 1-6.
5. Newmann DJ, Cragg CM. Natural product as source of new drug over last 25 years. J Nat Prod. 2007;70:461-477.
6. Hall JB. Ecology of a key African multipurpose tree species *Balanites aegyptiaca* Del. The state of knowledge. Forest Ecological Management. 1992;50: 1-30.
7. Hall JB. Ecology of a key African multipurpose tree species Balanites aegyptiaca Del. The state of knowledge. Forest Ecol Manag. 1992;50:1-30.
8. Chothani D L and Vaghasiya HU. A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacogen Rev. 2011;5(9):55-62.
9. Mansour HA, Newairy AA. Amelioration of impaired renal function associated with diabetes by *Balanites aegyptiaca* fruits in streptozotocin-induced diabetic rats. J Med Res Inst. 2000;21:115-125.
10. Motaal AA, Shaker S, Haddad PS. Antidiabetic activity of standardized extract of *Balanites aegyptiaca* Fruits using cell-based bioassays. Parmacognosy Journal. 2012;4(30):20-24.
11. Samir AM, Zaahkouk S, Rashid ZA, Mattar AF. Anti – diabetic properties of water and ethanolic extract of Balanites aegyptiaca fruits flesh in senile diabetic rats. Egyptian Journal of Hospital Medicine. 2003;10: 90-108.
12. George DH, Ali HK, El Abbas OA. Evaluation of the biological activity of *Balanites aegyptiaca* Del Saponin in the control of type 11 diabetes mellitus on rats and the growth of Escherichia coli. Ahfad J. Women Change. 2006;23:2.
13. Eman Helal GE, Abd El-Wahab SM, El Refaey H, Mohammad AA. Antidiabetic

and antihyperlipidemic effect of *Balanites aegyptiaca* Seeds (Aqueous Extract) on diabetic rats. The Egyptian Journal of Hospital Medicine. 2013;52:725–739.

14. Ezzat SM, Abdel Motaal A, El-Awdan SAW. *In vitro* and *in vivo* antidiabetic potential of extract and a furostanol saponin from *Balanites aegyptiaca*. Pharmaceutical Biology. 2017;55(1):1931-1936.
15. Gad MZ, El-Sawalhi MM, Ismail MF, El-Tanbouly ND. Biochemical study of the anti-diabetic action of the Egyptian plants *Fenugreek* and *Balanites*. Molecular and Cellular Biochemistry. 2006;281:173–183.
16. Shafik NH, Shafek RZ, Michael HN, Eskander EF. Phytochemical study and antihyperglycemic effects of *Balanites aegyptiaca* kernel extract on alloxan induced diabetic male rats. Journal of Chemistry and Pharmacy Research. 2016; 8(3):128-136.
17. Mahdy E, El-Sayed M. Antidiabetic effect of *Balanites aegyptiaca* leaves extract by regulation of erythrocyte glucose uptake in diabetic patient type 2 *in vitro*. Egyptian Journal of Hospital Medicine. 2017;67(2): 525-535. Abstract.
18. Gawade B, Farooqui M. Investigation of phytochemical and alpha amylase inhibition activity of *Balanites aegyptiaca* leaves. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018; 9(1):459-464.
19. Abubakar US, Adbullahi S, Ayuba V, Kaigama S, Halidu US, Ayuba MK. Medicinal plants used for the management of diabetes mellitus in Zaria, Kaduna State, Nigeria. Journal of Pharmacy and Pharmacognosy Research. 2017;5(3): 156-164.
20. Jung MY, Jeon BS, Bock JY. Free, esterified and insoluble bound phenolic acids in white and red ginsengs (*Panax ginseng* C.A. Meyer). Food Chem. 2002; 79:105–111.
21. Govorko D, Logendra S, Wang Y, Esposito D, Komarnytsky S, David R. Polyphenolic compounds from *Artemisia dracunculus L.* inhibit PEPCK gene expression and gluconeogenesis in an H4IIE hepatoma cell line. Am J Physiol Endocrinol Metab. 2007;293:1503–1510.
22. National Institute of Health (NIH). Principles of Laboratory Animal Care. NIH Publication. 1985;No. 85-23 Revised.
23. Gajdosik A, Gajdosikova A, Stefek M, Navarova J, Hozova R. Streptozotocin-induced experimental diabetes in male wistar rats. Gen Physiol Biophys. 1999; 18:54-62.
24. Kolawale OT, Akanji MA. Effects of extract of leaves of *Newbouldia laevis* on the activities of some enzymes of hepatic glucose metabolism in diabetic rats. African Journal of Biotechnology. 2014; 13(22):2273-228.
25. Goward CR, et al. Enzymatic assay of glucokinase. Sigma Quality Control Test Procedure. 1986;1-4.
26. Hengartner H, Harris JI. Phosphofructokinase from *Bacillus stearothermophilus*. Federation European Biochemical Society Letter. 1975;5:282.
27. Majumder AL, Eisenberg F Jr. In: Biswas T, Lahiri Majumder A, Guha G, Thakurt A, Mukherjee KL. Fructose-1, 6bisphosphatase in human fetal brain and liver during development. J. Biosci. 1982; 4(2):167-173.
28. Chang HC, Lane MD. The enzymatic carboxylation of phosphoenolpyruvate. II. Purification and properties of liver mitochondrial phosphoenolpyruvate carbooxykinase. Journal of Biochemistry. 1966;241:2413-2420.
29. Deutsch J. Glucose-6-phosphate dehydrogenase In: Bergmeyers Methods in Enzymatic Anal., 3rd Edition Beach, FL: Verlag Chemie. 1989;190.
30. Morgan HE, Parmeggiani A. Regulation of glycogenesis in muscle: Control of muscle glycogen phosphorylase activity. Journal of Biological Chemistry. 1964;238(8):2440-2445.
31. Baginski ES, Foa PP, Zak B. Glucose-6phosphatase. In: Methods of enzymatic analysis. (Ed. Bergmeyer HU). New York: Verlag Chemie Weinheim. Acad. Press Inc. 1974;737-764.
32. Danforth WH. Glycogen synthase assay. Journal of Biological Chemistry. 1965;240: 588.
33. Pogson CI, Denton RM. Effect of alloxan diabetes, starvation and refeeding on glycolytic kinase activities in rat epididymal adipose tissue. Nature. 1967;216:156-157.
34. Mhya DH, Amigo KM, Umar IA, Alegbejo JO. Evalaution of hypoglycemic potential of extracts of *Balanites aegyptiaca* parts. Int. J of Innovative and Advanced Studies. 2016;3(9):135-138.

35. Beach EF, Turner JJ. An enzymatic methods for glucose determination in body fluids. Clin. Chem. 1958;4:462-465.
36. Clark PM, Hales CN. How to measure plasma insulin. Diabetes Mellitus Metabolic Reserse. 1994;10(2):79-90.
37. Fossati P, Prenape L. Serum triglycerides determined colorimeterically with enzyme that produce hydrogen peroxide. Clin. Chem. 1982;28:2077-2080. 43.
38. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum, Z. Kin. Chem. Klin. Biochem. 1974;12(5):226-227.
39. Lopes-Virella MF, Stone S, Ellis S, Collwell JA. Cholesterol determination in highdensity lipoproteins separated by three different methods. Clin. Chem. 1977; 23:882-886.
40. Marchell WJ. Estimation of the concentration of low-density lipoprotein cholesterol in plasma. In Clinical Chemistray, 1992; 2nd ed., Gower Medical Publishing, UK. 1992;222-236.
41. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72:248-254.
42. Doumax BT, Watson WA HG. Clinical Chemistry, Acta, 1971;31:87.
43. Duncan BD. Multiple range test for correlated and heteroscedastic means. Biometrics. 1957;13:359–364.
44. Mhya DH, Anigo KM, Umar, IA and Alebejo, JO. Mechanisms and Antidiabetic Compounds of *Balanites aegyptiaca* delile Plant: A mini-review. South Asian Research Journal of Natural Products. 2018;1(2): 1-9.
45. Salwa AM, El Hadidi MN. Flavonoids of *Balanites aegyptiaca* (Balanitaceae) from Egypt. Plant System Evolution. 1988; 160(3):153–158.
46. Sarker SD, Bartholomew B, Nash RJ. Alkaloids from *Balanite aegyptiaca*. Fitoterapia. 2000;71:328–330.
47. Abdulhamid A, Sani I. Preliminary phytochemical screening and antimicrobial activity of aqueous and methanolic leave extracts of *Balanites aegyptiaca* (L). International Research Journal of Pharmaceutical and Biosciences. 2016; 3(1):1-7.
48. Bastaki S. Diabetes mellitus mellitus and its treatment. International Journal of Diabetes Mellitus Method. 2005;13: 111-134.
49. Karthikesan K, Pari L, Menon VP. Antihyperlipidemic effect of chlorogenic acid and tetrahydrocurcumin in rats subjected to diabetogenic agents. Chemico-Biological Interactions. 2010; 188(3):643–650.
50. Prince PSM, Kannan NK. Protective effect of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats. Journal of Pharmacy and Pharmacology. 2006;58(10):1373–1383.
51. Rydgren T, Sandler S. The protective effect of simvastatin against low dose streptozotocin induced type 1 diabetes in mice is independent of inhibition of HMG-CoA reductase. Biochemical and Biophysical Research Communications, 2009;379(4):1076–1079.
52. Aderibagbe AD, Adeghate E, Sharm AK, Pallot DJ, Singh J. Antihyperglycemic effect of *Mangifera indica* in rats. Phytother Res. 1999;13(6):504-507.
53. Gopalakrishnan G, Dhanapal CK. Evaluation of anti-diabetic activity of methanolic extract of *Coleus vettiveroides* Jacob in Streptozotocin-induced Diabetic rats. J. Pharm. Sci. & Res. 2014;6(2):97-103.
54. Unno T, Tago M, Suzuki Y. Effect of tea catechins on postprandial plasma lipid responses in human subjects. British Journal of Nutrition. 2005;93(4):543–547.
55. Sugiyama H, Akazome Y, Shoji T. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. Journal of Agricultural and Food Chemistry. 2007; 55(11):4604–4609.
56. Mauer SM, Steffes MW, Brown DM. The kidney in diabetes. American Journal of Medicine. 2007;70:63-6.
57. Qusti SY, Sharashili YR, Moselhy SS. Role of *Balanites aegyptiaca* in attenuation of Diabetic nephropathy. 2015;3(4):8-14.
58. Almdal TP, Vilstrup H. Effect of streptozotocin-induced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia. 2005;30:952-6.
59. Srinivasan S, Sathish G, Jayanthi M, Jayanthi M, Muthukumaran J, Muruganathan J. Ameliorating effect of *eugenol* on hyperglycemia by attenuating

the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. Molecular and Cellular Biochemistry. 2014;385(1-2):159-168.

60. Murphy ED, Anderson JW. Tissue glycolytic and gluconeogenic enzyme activities in mildly and moderately diabetic rats: Influence of Tolbutamide Administration Endocrinology. 1974;94(1): 27-34.
61. Latha M, Pari L. Antihyperglycemic effect of *Cassia auriculata* in experimental diabetes mellitus and its effects on key metabolic enzymes involved in carbohydrate metabolism. Clinical Experiment in Pharmacology and Physiology. 2003;30(1-2):38-43.
62. Soliman MM, Ahmed MM, El-Shazly SM. Cinnamon extract regulates gene expression of lipid and carbohydrate metabolism in streptozotocin induced diabetic wistar rats. American Journal of Biochemistry and Biotechnology. 2013; 9(2):172-182.
63. Gardiner NJ, Wang Z, Luke C, Gott A, Price SA, Fernyhoug P. Expression of hexokinase isoforms in the dorsal root ganglion of the adult rat and effect of experimental diabetes mellitus. Brain Reserve. 2007;1175:143–154.
64. Silva DD, Zancan P, Coelho WS, Gomez LS, Sola-Penna M. Metformin reverses hexokinase and 6-phosphofructokinase inhibition in skeletal muscle, liver, and adipose tissue from STZ-induced diabetic mouse. Archive of Biochemistry and Biophysics. 2010;496:53-60.
65. Nachar A, Vallerand D, Musallam S, Lavoie L, Badawi A, Arnason J. The action of antidiabetic plants of the Canadian James Bay Cree traditional pharmacopeia on key enzymes of hepatic glucose homeostasis. Evidence-Based Complementary and Alternative Medicine. 2013;1-9.
66. Deepak KGK, Nageswara R, Neelapu R, Surekha C. Role of antidiabetic compounds on glucose metabolism – a special focus on medicinal plant: *Salacia* sps. Medicinal Chemistry. 2014;4(3):373-381.
67. Jang SM, Kim MJ, Choi MS, Kwon EY, Lee MK. Inhibitory effects of ursolic acid on hepatic polyol pathway and glucose production in streptozotocin-induced diabetic mice. Metabolic Clinical Experiment. 2010;59(16):512–519.
68. Ramachandran V, Saravanan R. Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Phytomedicine. 2013;20(3-4):230–236.
69. Gutierrez RMP. Evaluation of the hypoglycemic and hypolipidemic effects of triterpenoids from *Prosthechea michuacana* in STZ-induced type 2 diabetes mellitus in mice. Pharmacologia, 2013;4:170–179.
DOI: 10.5567/pharmacologia2013.170.179
70. He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell. 2009; 137(5):635–646.
71. Skim F, Lazrek HB, Kaaya A, El-Amri H, Jana M. Pharmacological studies of two antidiabetic plants: *Globulria alypun and Zypophyllum gaetulumm*. Therapie. 1999; 54(6):711-715.
72. Birudu RB, Naik MJ, Janardhan M. Ethanolic extract of *Passiflora foetida* and silver nanoparticles on carbohydrate metabolic enzymes of dextrose induced diabetic rats. Journal of Biochemistry Biopharmacy and Biomedical Science. 2015;1(1):12-19.
73. Al-Malki AL, Barbour EK, Abullnaja KO, Moselhy SS. Management of hyperglycemia by ethyl acetate extract of *Balanites aegyptiaca* (Desert Date). Molecules. 2015;20(8):14425-14434.

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