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Effects of Aqueous Fraction of Ethanolic Extract of *Balanites aegyptiaca* Stem-bark on Glucose Metabolic Enzymes in Streptozotocin-induced Diabetic Rats

D. H. Mhya[1*], K. M. Anigo[1], I. A. Umar[1] and J. O. Alegbejo[2]

[1]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Pediatric, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Authors' contributions

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

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Original Research Article

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ABSTRACT

Aims: The study examines effect of aqueous-fraction of ethanolic extract of *Balanites aegyptiaca* Stem-bark on enzymes of glucose metabolism in streptozotocin (STZ) diabetic rats in a bid to understand its antihyperglycemic mechanism of action.
Methodology: Diabetes was induced in male rats by intra-peritoneal injection of 60 mg/kg body weight of STZ. *Balanites aegyptiaca* stem-bark was extracted using ethanol followed by solvent-

**Corresponding author: E-mail: dmhyas@gmail.com;*

solvent fractionation with ethyl acetate and water. The aqueous fraction obtained was subjected to acute toxicity on wistar rats using a gradient dosage, 1/10th of lethal dose was calculated and used for the study. It was orally administered at a dose of 400 mg/kg body wt to diabetic rats, metformin (200 mg/kg body wt) serve as reference drug and diabetic/normal controls received 10% dimethyl sulfurdioxide (DMSO) for the 28 days treatment period. On day 29th, rats were sacrificed; blood and liver samples were collected. Liver tissues were homogenized, centrifuged and the supernatants were used for assay of glucose metabolic enzymes while serum was used for biochemical markers estimations.

Results: Results obtained showed no death or lethal effect in the acute toxicity study up to a dose of 4000 mg/kg bwt therefore, the LD_{50} value was considered to be more than 4000 mg/kg body wt. When the streptozotocin induced diabetic rats were treated with aqueous-fraction of the stem-bark ethanolic extract a significant (*P≤0.05*) regulation in glucose metabolic enzymes were noticed; glucokinase activity increased (3.04±0.004 U/min/mg protein) against diabetic control (2.22±0.016 U/min/mg protein) as well as glycogen synthase (0.13±0.001 U/min/mg protein) against diabetic control (0.09±0.003 U/min/mg protein). Glucose-6-phosphatase activity decreased (0.26±0.028 U/min/mg protein) against untreated diabetes (1.44±0.0.054 U/min/mg protein). Glycogen content increased to 13.77±0.32 mg/g liver compared to diabetic control (10.69±0.32 mg/g liver). A significant effect on fasting blood glucose was observed; treated diabetes (290.4±18.4 mg/dL) compared to diabetic control (336.0±11.9 mg/dL).

Conclusion: These results indicated that *Balanites aegyptiaca* stem-bark contained compound(s) that regulates glucose metabolic enzymes to achieved its antihyperglycemic effect.

Keywords: Balanite aegyptiaca; stem-bark; aqueous-fraction; metabolic-enzymes.

1. INTRODUCTION

Diabetes is a key health problem worldwide with an increase frequency rate [1]. It is a metabolic disorder of multiple etiologies, which is characterized by hyperglycemia caused by defects or alterations in either secretion or action of insulin. Diabetes causes derangement of several metabolisms including carbohydrate metabolism which has significant influences on glucose homeostasis [2,3]. Diabetes has been shown to depress the activities of glycolytic enzymes while promoting gluconeogenic enzymes activities [4].

In this study, we used streptozotocin for our experiment in induction of experimental diabetes mellitus. The diabetogenic agent streptozotocin inhibits insulin secretion and caused a state of insulin-dependent diabetes mellitus through its ability to induce a selection of necrosis of the pancreatic beta cell [5]. Common options for medical hyperglycemic control is the used of oral antidiabetic drugs, the effect of these drugs is aimed to lower the level of blood glucose [6]. However, this drug may be effective for glycemic control, but has side effects such as liver disorders, flatulence, abdominal pain, and diarrhea [7]. In addition, most of hypoglycemic agents or drugs are not effective to decrease blood glucose levels in chronic diabetic patients [6]. As a result many patients resort to herbal products as alternative medicine. The World Health Organization has recommended that treatments for diabetes mellitus, using plants warrant greater attention [8].

The underlying goal of all diabetes treatments or management is to maintain normal glycemia. Maintenance of a normal plasma glucose concentration requires precise matching of glucose utilization and endogenous production. This could be achieved by the regulation of two major metabolic pathways, gluconeogenesis and glycogenolysis, which produce glucose in the liver [9]. In addition, the key enzymes in opposing metabolic pathways such as glycolysis and glycogenesis must also be regulated in order for net flux in the appropriate direction to be achieved. Agius [10] have shown that inhibition of enzymes involved in gluconeogenesis and/ or glycolgenolysis suppresses hepatic glucose production and lower fasting plasma glucose. Several plants like *Plumbago zeylanica* and *Citrus unshiu* have showed significant impact on the activities of glucose metabolizing enzymes in diabetes [11,12]. Farsi et al. [13] have reported that *Ficus deltoidea Jack*. (Moraceae) retards phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase activities. Similar finding have been reported on *Newbouldia leavis* extract by Kolawole et al. [14].

Research on medicinal plants has led to the isolation and identification of a number of bioactive compounds like polyphenolics [15]. Polyphenolics from plant origin have been used to treat human and animal diseases due to their effectiveness, safety and lesser side effects [16]. For instance, pl'nts' compounds such as; tannic acid, quercetin, catechin, epicatechin, and ferulic acid and coumarin" and "quercetin derivatives", respectively were reported to have significantly regulation on glucokinase and glycogen synthesis in diabetic rats [17,18]. Also, *eugeno*l isolated from Cloves and *fisetin* (a bioflavonoid found in fruit and vegetable) were reported to 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 [19,20].

The plant '*Balanites aegyptiaca Del*.', 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 [21,22]. In Nigeria, it is found in abundant in the Northern region. It is known as '*Aduwa*' in Hausa, '*Utazi*' in Igbo, and '*Teji*' in Yoruba. Literature survey revealed that *Balanites aegyptiaca* has a long history of traditional uses for wide ranges of disease including diabetes [23]. Anti-diabetic activities of *B. aegyptiaca* fruit have been reported in both diabetic mice and rats [24,25]. Mansour and Newairy [26] have also reported antidiabetic effect of aqueous extract of *Balanites aegyptiaca* on streptozotocin-induced diabetic mice. Phytochemical investigation of *Balanites aegyptiaca* parts revealed the presence of various polyphenolics; coumarins and quercetins were found in the leaves, alkaloids and coumarins were seen in the stem-bark while rutins was found in the fruit among others [27,28]. It was reported that hypoglycemic activity of *Balanites aegyptiaca* may be via enhancement of peripheral glucose metabolism and an increase in insulin release [29]. Other study suggested antioxidant potential to combat diabetes [30]. However, there is no data reporting the effect of *Balanites aegyptiaca* on glucose enzymatic modulation and investigation on antidiabetic activity of the plant's stem-bark is scanty. Hence, the need to examine effect of the plant (*Balanites aegyptiaca)* stem-bark on glucose metabolic enzymes in streptozotocin (STZ)-induced diabetic rats in a bid to understand its antihyperglycemic activity.

2. MATERIALS AND METHODS

2.1 Plant Collection

Balanites aegyptiaca stem-bark was collected from Gubi village in Bauchi, Bauchi state. It was identified and assigned a voucher number 900175 at the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University Zaria.

2.2 Extraction

Extraction: Extract was prepared as done by Jung et al. [31] and Govorko et al. [32]. Plant sample powdered (50 g) was defatted twice for 2 hrs with 80 mL hexane on a mechanical shaker. The hexane solvent was discarded, the defatted sample powder was air-dried and then, 10 g of the defatted powdered sample of the *Balanite aegyptiaca* was heated to 80℃ with 100 ml of 80% ethanol for 2 hrs. The extraction was continued for an additional 10 hrs at 20℃. The extract was filtered through a cheese cloth and concentrated by evaporation to 10 ml using a rotary evaporator. The ethanolic extract was partitioned with 10 ml of water and 10 ml of ethyl acetate in a separating funnel, the aqueous phase was carefully collected and concentrated using a rotary evaporator and finally air dried. The dried aqueous fraction was then used for the study.

2.3 Chemicals/Reagents

All chemicals/reagents used were of analytical grade and were obtained from Sigma Aldrich, USA. Reagent kits were purchased from Randox Laboratory, UK.

2.4 Animals

Twenty (20) male wistar albino rats, approximately of the same age weighing between 200-230 g purchased from the Department of Biochemistry, University of Jos, Plateau state were used for the study. They were allowed free access to water and animal feed (Vital feeds, Jos).

2.5 Animals' Grouping

Animals grouping: Rats were allocated into four (4) groups of 5 rats each as follows:

Group A: Diabetic rats received aqueous fraction of *B. aegyptica* Stem-bark (400 mg/kg body wt).

Group B: Diabetic rats received Metformin, (200 mg/kg body wt).

Group C: Diabetic control rats (received 10% DMSO).

Group D: Normal control rats (received 10% DMSO).

2.6 Experimental Design

The aqueous fraction of ethanolic extract of *Balanite aegyptiaca* stem-bark was assessed for acute toxicity, phenolics and flavonoids content was quantified. Thereafter, antihyperglycemic effects of aqueos fraction of ethanolic extract of *Balanites aegyptiaca* stem-bark was investigated in Streptozotocin-induced diabetic rats for 28 days. On day 29th, At the end of the experiment, rats were sacrificed by humane decapitation and blood was collected and allow to cloth at 25℃ thereafter serum was collected and used for biochemical markers estimations. Liver were excised, homogenized and centrifuged using refrigerated centrifuge 10,000 x g at 4℃, the supernatant was used for assaying the activities of hepatic key enzymes of glucose metabolism.

2.7 Phytochemical Analysis

Qualitative phytochemical analysis of plant extract was carried out by using standard procedures to identify the constituents as done by Edeoga et al. [33].

2.8 Determination of Total Flavonoids (TFC) and Phenolics Contents (TPC)

The TFC of the extract-fraction of *Balanites aegyptiaca* stem-bark was determined using the aluminium trichloride [34]. Folin-Ciocalteu method was used to determine the total phenolics content of the aqueous fraction of ethanolic extract of *Balanites aegyptiaca* stem-bark Singleton et al. [35].

2.9 Acute Toxicity Study

The acute toxicity study was performed according to OECD 425 guidelines: up-and-down acute toxicity test [36]. The extracts were administered in a single dose by using oral gastric tube. Animals were deprived of food 3 hours prior to dosing, after each extract administration animal was observed 30 minutes interval for 4 hours then after 24 hours for behavioral change or death. The Following dosage (5, 50, 500, 1000, 2000, 4000 mg/kg body wt) were determined and used according to the OECD 425 guidelines with limit at 2000-5000 mg/kg body wt. Bruce's [37] up and down procedure for oral acute toxicity study was used to investigate acute toxicity of the plant extract on wistar albino rats. Lethal Median Dose (LD_{50}) was calculated using the formula below: LD_{50} = (the apparent least dose lethal to animals – [(a x b)/N]).

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

NB: For the study, 1/10th of LD_{50} was used.

2.10 Induction of Diabetes Mellitus

Type I diabetes was induced in rats by intra-peritoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body wt in 0.1 M citrate buffer (pH 4.5). Rats were supplied with 5% 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.

2.11 Estimation of Biochemical Markers

The following biochemical makers were determined following a standard procedures: Blood glucose [38], Hepatic glycogen Content [39], Fructosamine [40], Total Protein) [41], Plasma triglyceride (TG): [42], Total Cholesterol: [43], High Density Lipoprotein Cholesterol (HDL-C) [44], Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) [45]. Hepatic key glucose metabolic enzymes like Glucokinase activity [46], Phosphofructokinase (PFKinase) activity [47], Fructose-1,6-bisphosphatase activity [48], Phosphoenolpyruvate carboxylkinase activity [49], Glycogen phosphorylase activity [50], Glucose-6-phosphatase (G6Pase) activity [51], Glycogen synthase activity [52], Lactate Dehydrogenase (LDH) [53], Pyruvate kinase (PK) [54].

2.12 Data Analysis

The results of the experiments were pooled and 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) [55]. Significant difference was accepted at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The anti-diabetic effect of *Balanites aegyptiaca*, a known medicinal plant has been reported by several studies, particularly in Africa [22,23]. However, it remains yet to be known with certainty the effective agent responsible for the anti-diabetes and the mechanism of action. In our study, anti-diabetic activity of extract prepared from the stem-bark of the plant was studied on the activities of key enzymes of glucose metabolism in liver of streptozotocin (STZ) diabetic rats in order to understand in part the glucose lowering mechanism of this medicinal plant.

3.1 Phytochemical Constituent of Aqueous Fraction of Ethanolic Extract of *Balanites aegpytiaca* Stem-bark

The chemical constituents of aqueous fractions of ethanolic extract of *Balanites aegyptiaca* stem bark shows the presence of tannins, flavonoids, phenolics, and saponins. The medicinal value of plants lies in some chemical substances known as phytochemicals that have a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, saponins, terpenoids, steroids, glycosides, flavanoids and phenolics [56]. Phytochemical screening of *Balanites aegyptiaca* stem-bark revealed the presence of some of these important chemicals confirming its usefulness in disease intervention. Phytochemical investigation of *Balanites aegyptiaca* parts showed the presence of various polyphenolics; coumarins and quercetins were found in the leaves, alkaloids and coumarins were seen in the stem-bark while rutins was found in the fruit among others [27,28].

3.2 Acute Toxicity Study of Aqueous Fraction of Ethanolic Extract of *Balanites aegpytiaca* Stem-bark

From the experiment performed as per the OECD Guidelines 425 for up-and-down acute toxicity test, the results reveal that aqueous fractions of ethanolic extract of *Balanites aegyptiaca* stem-bark was safe up to a dose of 4000 mg/kg body wt. Observation made 4 hours and later 24 hours after administration shows; no treatment-related mortality at all the tested doses, no significant changes in behavior such as apathy, hyperactivity, morbidity, etc. recorded in the treated animals. The aqueous fractions derived from ethanolic extract of *Balanites aegyptiaca* stem-bark was safe up to a dose of 4000 mg/kg body wt and therefore, the LD_{50} value for oral toxicity is considered to be greater than 4000 mg/kg body wt.

3.3 Phenolics and Flavonoids Content of Aqueous Fraction of Ethanolic Extract of *Balanites aegyptiaca* Stem-bark

Total phenolics content was (0.52±0.0416 mg g^{-1}Garlic acid Equivalent) and total flavonoids was (0.04±0.0001 mg g^{-1}Quercetin Equivalent) in the aqueous fraction of ethanolic extract of *Balanites aegyptiaca* Stem-Bark. Results indicated that phenolics compounds are high in aqueous fraction of the extract. It was noted that water enhances interaction of hydroxyl and or carboxylic groups, hence promote dissolution of phenolics in the solvent [57]. On the other hand, flavonoids have low solubility in water and this suggest its low quantity in the aqueous fraction.

3.4 Antihyperglycemic Effect of Oral Administration of Aqueous Fraction of Ethanolic Extract of *Balanites aegyptiaca* Stem-bark in Streptozotocin-induced Diabetic Rats

The effect of aqueous fractions of ethanolic extract of *Balanites aegyptiaca* stem-bark on blood glucose in STZ diabetic rats is given in Table 1. In the diabetic control rats, there was a peak increase in fasting blood glucose level which continues throughout the experimental period. However, fall in fasting blood glucose level in diabetic rats treated with metformin and aqueous fractions of ethanolic extract of *Balanites aegyptiaca* stem-bark were observed and are significant ($P \leq 0.05$) when compared to diabetic control. The glucose lowering effect of aqueous fractions of ethanolic extract of *Balanites aegyptiaca* stem-bark might be time-dependent suggesting that better effect may be achieved with prolong treatment period.

3.5 Effect of Aqueous Fraction of Ethanolic Extract of *Balanites aegpytiaca* Stem-bark on Some Biochemical Markers in Streptozotocin Diabetic Rats

The result of hepatic glycogen content, serum albumin, fructosamine, total protein and lipid

profile following treatment of STZ diabetic rats with *Balanites aegyptiaca* stem-bark is presented in Table 2. There was a significant reduction (*P≤0.05*) in glycogen content of untreated diabetic rats (10.69±0.32 mg/g Liver) in comparison to the diabetic treated rats (13.77±0.32 mg/g Liver) and metformin-treated diabetic rats (17.77±0.32 mg/g Liver) were observed. It was stated that glycogen levels in liver tissues decreases as the influx of glucose in liver is inhibited in the absence of insulin and recovers on insulin treatment [58,59]. From the present study, there was a significant reduction of glycogen content in liver of untreated diabetic animals; the decreased glycogen content may result from insulin deficiency in diabetic state that leads to low activity of glycogen synthase in the liver. A significant increased in glycogen content of rats received *Balanites aegyptiaca* stem-bark extract is probably due to activation of glycogen synthase activity. Similar activities by plants extract on the enzyme (glycogen synthase) and hepatic glycogen content in diabetic treated rats have been reported [60,61].

STZ induced diabetic rats showed significant increase in cholesterol, TG, VLDL and decrease in HDL compared to normal and diabetic treated rats. Administration of extract of *Balanites aegyptiaca* stem-bark significantly restored their levels. Serum cholesterol level in diabetic rats that received metformin (180.61±3.19 mg/dL) and aqueous fraction of ethanolic extract of the Plant stem-bark (172.00±2.96 mg/dL) were lowered compared to diabetic control value (232.00±2.96 mg/dL). Similarly, elevated serum triglycerides levels were significantly (P≤0*.05*) reduced in the diabetic rats received stem-bark extract (129.83±3.32 mg/dL) against diabetic control (207.13±6.05 mg/dL). Regulation in the lipids profile could suggest ability of the plant extract to reduce complications accompany diabetes. The findings is consisted to the report by Samir et al. [23] that showed a decrease of serum total cholesterol and triglycerides in STZ diabetic rats treated with aqueous and ethanolic extracts of *Balanites aegyptiaca* fruit.

It has been reported that plants extract exert their cholesterol lowering effect by decreasing in cholesterol absorption from the intestine, by binding with bile acids within the intestine and increasing bile acids excretion [62]. A significant decrease in serum cholesterol observed in diabetic rats received plant extract in our experiment might in part agreed with the above statement. In another dimension, one could suggest inhibition of Hydroxyl Methyl Glutaryl A Co-enzyme reductase (HMG-CoA reductase) activity by the plant, since study has shown an increased HMG-CoA reductase activity in diabetic rats [63]. It have also been reported that, hyper-triglyceridemia characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of pancreatic lipase [64]. Diabetes mellitus results in failure to deactivate this enzyme thereby casing hyper-triglyceridemia. From our study, it is assumed that the administration of *Balanites aegyptiaca* extract 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 [65,66].

It was shown that glycogen levels in liver tissues decreases as the influx of glucose in liver is inhibited in the absence of insulin and recovers on insulin treatment [58]. In this study, there was a significant reduction of glycogen content in liver of untreated diabetic animals; the decreased glycogen content may result from insulin deficiency in diabetic state that leads to low activity of glycogen synthase in the liver. A significant increased in glycogen content in rats that received *Balanites aegyptiaca* extract was observed and this is probably due to activation of glycogen synthase activity. Increased in hepatic glycogen content as a result activation of glycogen synthase by plant extract in diabetic rats has been reported by Jang et al. [59] and Ramachadran and Saravanan [60]. Low level of fructosamine in diabetic treated rats in this study suggests glycation inhibitory effect of aqueous fraction of the ethanolic extract of *Balanites aegyptiaca* stem-bark.

3.6 Effect of Aqueous Fraction of Ethanolic Extract of *Balanites aegpytiaca* Stem-bark on Glucose Metabolic Enzymes in Streptozotocin Diabetic Rats

Activities of key glucose metabolic enzymes were determined in liver tissues of STZ-induced diabetic rats following intervention with aqueous fraction of *Balanites aegyptiaca* stem-bark, and the standard metformin (Table 3). Significant changes were observed in the activities of glycolytic enzymes like glucokinase (3.04±0.004 U/min/mg protein) against untreated diabetic

(2.22±0.016 U/min/mg protein), gluconeogenic enzymes like Glucose-6-phosphatase (0.26±0.028 U/min/mg protein) against untreated diabetic (1.44±0.0.054 U/min/mg protein), and glycogen synthase (0.13±0.001 U/min/mg protein) against untreated diabetic (0.09±0.003 U/min/mg protein) etc.

Liver functions as a "glucostat". Its plays a vital role in the maintenance of blood glucose level and hence it is of interest to examine the possible role of *Balanites aegyptiaca* stem-bark extract on hepatic key enzymes of carbohydrate metabolism. Results of our studies indicate low activities of hepatic glycolytic enzymes in STZ diabetic animals. Impairment of glycolytic enzymes activity suggests impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia. Activities of glucolytic enzymes are very sensitive signs of glycolytic pathway and these are decreased in the liver of diabetic state [67]. The decrease of the activities of glycolytic enzymes in STZ-induced diabetic rats in our study was consistent with other studies on glycolytic enzymes [4,68]. However, administration of aqueous fraction of ethanolic extract of *Balanites aegyptiaca* stem-bark to diabetic rats stimulate these enzymes supporting the notion that part of the therapeutic potential of several anti-diabetic plants involves modulation of glucose metabolic enzymes activities [69].

Table 1. Antihyperglycemic effect of oral administration of aqueous fraction of ethanolic extract of *Balanites aegyptiaca* stem-bark in streptozotocin-induced diabetic rats

	Fasting blood glucose levels (mg/dL)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Diabetic + AFS	247.0±13.8^{a}	272.6±18.5^{b}	305.6±22.1^{bcd}	300.0±22.1^{bcd}	290.4±18.4^{bc}
Diabetic + Metformin	242.8±10.0^{b}	274.2±15.7^{bc}	269.8±19.6^{bc}	243.0±16.2^{b}	182.6±1.12^{a}
Diabetic control	248.8±07.5^{a}	282.6±09.8^{b}	301.4±10.9^{bc}	317.6±08.7^{bc}	336.0±11.9^{bcd}
Normal control	67.6±02.0^{a}	77.2±10.8^{a}	93.6±11.5^{b}	94.4±08.7^{b}	96.0±02.8^{b}

Values are Mean ± Std of 5 determinants, values with different superscript in the same row are significantly different (P≤0.05)

Values designated (a) shows significant decrease, (b) shows significant increase, (bc) shows significant moderate increase, (bcd) shows significant high increase when compared among the groups

AFS = Aqueous Fraction of Ethanolic extract of B. aegyptiacia Stem-bark

Table 2. Effect of aqueous fraction of ethanolic extract *of Balanites aegyptiaca* stem-bark on some biochemical markers of streptozotocin diabetic rats

	Diabetic + AFS	**Diabetic control**	**Diabetic + Metformin**	**Normal control**
Glycogen (mg/g liver)	13.77±0.322^{b}	10.69±0.320^{a}	17.77±0.320^{bc}	15.85±0.320^{bc}
Fructosamine (mmol/L)	0.09±0.013^{b}	0.50±0.159^{bc}	0.08±0.004^{b}	0.05±0.028^{a}
Albumin (g/dL)	2.57±0.005^{b}	2.21±0.159^{a}	3.59±0.004^{bc}	4.28±0.005^{bcd}
Total protein (mg/g liver)	122.3±0.843^{b}	97.3±0.436^{a}	148.0±0.272^{bc}	159.5±0.230^{bcd}
Cholesterol (mg/dL)	212.31±2.34^{bc}	232.00±2.96^{bcd}	180.61±3.19^{b}	76.31±3.19^{a}
Triglyceride (mg/dL)	129.83±3.32^{bc}	207.13±6.05^{b}	99.91±5.54^{a}	97.82±2.90^{a}
HDL-Cholesterol (mg/dL)	39.51±0.87^{b}	26.91±5.97^{a}	66.35±5.52^{bc}	47.78±3.01^{b}
LDL-Cholesterol (mg/dL)	152.44±2.48^{bc}	172.39±4.69^{bcd}	147.36±4.57^{b}	47.19±3.56^{a}
VLDL (mg/dL)	45.97±0.66^{bc}	41.43±1.21^{b}	19.98±1.11^{a}	19.57±0.58^{a}

Values are Mean ± Std of 5 determinants, values with different superscript in the same row are significantly different (P≤0.05) Values designated (a) shows significant decrease, (b) shows significant increase, (bc) shows significant moderate increase, (bcd) shows significant high increase when compared among the groups

AFS = Aqueous Fraction of Ethanolic extract of B. aegyptiacia stem-bark

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose- 6-phosphatase, fructose -1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase [70]. Gluconeogenesis is the major metabolic pathway through which the liver produces glucose from precursors such as amino acids, lactate, glycerol, and pyruvate. The hepatic gluconeogenic enzymes studied were elevated significantly in diabetic rats. This may be due to the increased synthesis of the enzymes contributing to the rise in glucose production during diabetes by the liver [71,72]. The administration of *Balanites aegyptiaca* stem-bark extract retards the activities of these enzymes similar to what have been reported by some studies on several plants extract [13,73,74].

Glycogen synthase (GS) catalyzes the rate limiting step of glycogenogenesis and is thus responsible for the storage of glucose in the liver. *Balanites aegyptiaca* stem-bark extract effectively increased the activity of glycogen synthase, while phosphorylase activity was decreased in the treated diabetic rats when compared to the untreated diabetes. This is supported by the relatively higher glycogen content observed in the diabetic -treated rats. Similar activities of other plants extract on the selected glycogen enzymes and hepatic glycogen content in diabetic rats have been reported [54,62]. The observed activation of glycogen synthase along with inhibition of phosphorylase, an enzyme that catalyzes glycogenolysis further supports insulinogenic activity of the plant. Gutierrez [75] has also reported this insulinogenic character of Prosthechea michuacana in STZ-induced diabetic rats.

Table 3. Effect of aqueous fraction of *Balanites aegyptiaca* stem-bark ethanolic extract on glucose metabolic enzymes activities in liver of streptozotocin diabetic rats

	Diabetic + AFS	Diabetic control	Diabetic + metformin	Normal control
Glucokinase (U/min/mg protein)	3.04 ± 0.004^{bcd}	2.22 ± 0.016^{a}	2.72 ± 0.015^{b}	3.53 ± 0.006^{bc}
Phosphofructokinase (U/min/mg protein)	3.49 ± 0.011^{bc}	2.06 ± 0.074^{a}	3.34 ± 0.014^{b}	4.05 ± 0.075^{bcd}
Pyruvate kinase (U/min/mg protein)	0.01 ± 0.001^{b}	0.004 ± 0.002^{a}	0.02 ± 0.002^{bc}	0.01 ± 0.001^{b}
LDH U/min/mg protein	0.06 ± 0.016^{b}	0.04 ± 0.028^{a}	0.06 ± 0.016^{b}	0.07 ± 0.22^{bc}
Glycogen phosphorylase (U/min/mg protein)	3.14 ± 0.070^{b}	3.82 ± 0.210^{bc}	2.04 ± 0.010^{a}	2.07 ± 0.660^{a}
Glycogen synthase (U/min/mg protein)	0.13 ± 0.001^{b}	0.09 ± 0.003^{a}	0.16 ± 0.004^{bc}	0.29 ± 0.009^{bcd}
Glucose-6-Phosphatase (U/min/µmole P_i liberated)	0.26 ± 0.028^{bc}	$1.44\pm0.0.054^{bcd}$	0.12 ± 0.017^{b}	0.07 ± 0.012^{a}
Fructose-1,6-Bis-Phosphatase (U/min/µmole P_i liberated)	1.42 ± 0.088^{b}	2.14 ± 0.247^{bc}	1.02 ± 0.021^{a}	1.39 ± 0.074^{b}
Phosphoenol-pyruvate Carboxylase (U/min/mg protein)	0.25 ± 0.114^{bc}	0.72 ± 0.031^{bcd}	0.14 ± 0.028^{b}	0.08 ± 0.041^{a}
Pyruvate carboxylase (U/min/mg protein)	0.02 ± 0.003^{a}	0.12 ± 0.001^{bc}	0.03 ± 0.002^{b}	0.02 ± 0.006^{a}

Values are Mean ± Std of 5 determinants, values with different superscript in the same row are significantly different (P≤0.05)

Values designated (a) shows significant decrease, (b) shows significant increase, (bc) shows significant moderate increase, (bcd) shows significant high increase when compared among the groups

AFS = Aqueous Fraction of Ethanolic extract of B. aegyptiacia Stem-bark

4. CONCLUSION

It is concluded that aqueous fraction of ethanolic extract of *Balanites aegyptiaca* stem-bark at a dose of 400 mg/kg body wt exhibit anti-hypergylcemic effects in STZ diabetic rats perhaps via its influence on the impaired activity of enzymes in carbohydrate metabolism.

ETHICAL APPROVAL

All authors hereby declare that 'principle of laboratory animal care' was followed. The experiment have been examined and approved by the ethics committee for animals use in Ahmadu Bello Univiersity Zaria, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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