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Effects of Solvent Fractions of *Balanites aegyptiaca* Stem-bark Ethanolic Extract on Glucose Metabolic Enzymes in Diabetic Rats

ABSTRACT

Aims: This study investigates anti-hyperglycemic effect of aqueous-fraction of *Balanites aegyptiaca* Stem-bark ethanolic extract on enzymes of glucose metabolism in streptozotocin (STZ) diabetic rats.

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-solvent fractionation with ethyl acetate and water. The aqueous fraction obtained was used; it was orally administered at a dose of 400 mg/kg bwt (i.e.1/10th of lethal dose) to diabetic rats, metformin (200 mg/kg bwt) serve as reference drug and diabetic/normal controls received 10 % dimethyl sulfurdioxide (vehicle) 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 >4000 mg/kg bwt. When the streptozotocin induced diabetic rats were treated with aqueous-fraction a significant (*P=.05*) regulation in glucose metabolic enzymes were noticed; glucokinase activity increased (3.04±0.004 U/min/mg Protein) against untreated diabetes (2.22±0.016 U/min/mg Protein) as well as glycogen synthase (0.13±0.001 U/min/mg Protein) against untreated diabetes (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 untreated diabetes (10.69±0.32 mg/g liver). Significant effects on fasting blood glucose was observed; treated diabetes (290.4±18.4 mg/dL) and untreated diabetes (336.0±11.9 mg/dL).

Conclusion: These results indicated that *Balanites aegyptiaca* exhibited a significant potential as antihyperglycemic agent perhaps via its effects on glucose metabolic enzymes.

Keywords: *Balanite aegyptiaca,* stem-bark, aqueous-fraction, metabolic-enzymes

1.0 INTRODUCTION

Diabetes is a key health problem worldwide with an increase frequency rate [1]. It is a metabolic disorder of multiple etiologies, characterize by chronic 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].

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 achieve by the regulation of two major

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metabolic pathways, gluconeogenesis and glycogenolysis, which produce glucose in the liver [5]. 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 [6] have shown that inhibition of enzymes involves 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 [7,8]. Farsi *et al* [9] have reported that *Ficus deltoidea Jack*. (Moraceae) retards phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase activity on the other hand stimulate hepatic glucokinase (GK) in skeletal muscles of streptozotocin-induced diabetic rats. Similar finding have been reported on *Newbouldia leavis* extract by Kolawole *et al* [10].

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 [11,12]. 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 *B. aegyptiaca* has a long history of traditional uses for wide ranges of disease including diabetes [13]. Anti-diabetic activities of *B. aegyptiaca* have been reported in both diabetic mice and rats [14,15]. However, the investigations did not explorate on the probable antihyperglycemic action of the plant. This study attempt to investigates effect of aqueous-fraction of *Balanites aegyptiaca* Stem-bark ethanolic extract on key enzymes of glucose metabolism in liver of streptozotocin (STZ) diabetic rats in a bid to explore its probable antihyperglycemic action.

2. 0 MATERIALS AND METHODS

2.1 Plant Collection and Extraction

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. Extraction: Extract was prepared as done by Jung *et al* [16] and Govorko *et al* [17]. 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

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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 extract was then partitioned with 10 ml of water and 10 ml of ethyl acetate. The aqueous fraction ~~used~~ was concentrated using a rotary evaporator and air dried.

2. 2 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. 3 Animals/Grouping

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). 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 b wt)

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

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

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

2.4 Experimental Design

The polyphenols-rich *Balanite aegyptiaca* stem-bark extract was assessed for acute toxicity, phenols and flavonoids content was quantified. Thereafter, antihyperglycemic effects of the the polyphenols-rich *Balanites aegyptiaca* stem-bark extract was investigated in Streptozotocin-induced diabetic rats for 28 days. On day 29th, animals were sacrificed and liver were excised, homogenized and centrifuged using refrigerated centrifuge 10,000 x g at 4 ^{o}C, the supernatant was used for assaying the activities of hepatic key enzymes of glucose metabolism. Blood was collected and serum separated ~~and~~ used for biochemical estimations.

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2.5 Determination of Total Flavonoids (TFC) and Phenols Contents (TPC)

The TFC of the extract of *Balanites aegyptiaca* stem-bark was determined using the aluminium trichloride (18). Folin-Ciocalteu method was used to determine the total phenols content of the aqueous fraction of *Balanites aegyptiaca* stem-bark ethanolic extract (Singleton *et al* [19].

2.6 Acute Toxicity Study

Bruce's [20] up and down method for 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, $1/10^{th}$ of LD_{50} was used.

2.7 Induction of Diabetes Mellitus

Rats were made diabetic by intra-peritoneal injection of Streptozotocin (STZ) (60 mg/ml/kg bwt in citrate buffer, 0.1 M, 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.

2.8 Estimation of Biochemical Markers

The following biochemical makers were determined following ~~a~~ standard procedures: Blood glucose [21], Hepatic glycogen Content [22], Fructosamine [23], Total Protein)[24], Plasma triglyceride (TG): [25], Total Cholesterol: [26], High Density Lipoprotein Cholesterol (HDL-C) [27] ~~and~~, Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) [28] .Hepatic key glucose metabolic enzymes including Glucokinase activity [29], Phosphofructokinase (PFKinase) activity [30], Fructose-1,6-bisphosphatase activity [31], Phosphoenolpyruvate carboxylkinase activity [32], Glycogen phosphorylase activity [33], Glucose-6-phosphatase (G6Pase) activity [34]. Glycogen synthase activity [35], Lactate Dehydrogenase (LDH) [36], Pyruvate kinase (PK) [37].

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2.9 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) [38]. Significant difference was accepted at *P*=.05.

3.0 RESULTS AND DISCUSSION

The anti-diabetic effect of *Balanites aegyptiaca*, a known medicinal plant has been reported by several studies, particularly in Africa [13,14]. 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 in order to understand the glucose lowering mechanism of this medicinal plant by evaluating its impact on key hepatic enzymes of glucose metabolism in liver of streptozotocin (STZ) diabetic rats.

3.1 Effect of Aqueous Fraction of *Balanites aegpytiaca* Stem-Bark Ethanolic Extract 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 1. There was a significant reduction (*P=.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 [39,40]. 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 [41,42].

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*

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stem-bark significantly restored their levels. Serum cholesterol level in diabetic rats (232.00±2.96 mg/dL) significantly (*P=.05*) increased compared diabetic rats received metformin (180.61±3.19 mg/dL) and stem-bark extract (172.00±2.96 mg/dL). Similarly, elevated serum triglycerides levels were significantly (*P=.05*) reduced in the diabetic rats received stem-bark extract (129.83±3.32 mg/dL) against untreated diabetic (207.13±6.05 mg/dL). Regulation in the lipids profile could suggest ability of the plant extract to inhibit complications accompany diabetes.

3.2 Phenols and Flavonoids Content of stem-bark Extract of *Balanites aegyptiaca*

The determined amount of total phenols and total flavonoids content of aqueous fraction of *Balanites aegyptiaca* stem-bark ethanolic extract showed superior but varying level of total phenols and flavonoids. The total phenol~~s~~ content was (0.52±0.0416 mg g^{-1}GAE) and total flavonoids was (0.04±0.0001 mg g^{-1}QE) respectively. 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 [43]. On the other hand, flavonoids have low solubility in water and this suggest its low quantity in the aqueous fraction.

Table 1. Effect of Aqueous Fraction of *Balanites aegyptiaca* Stem-Bark Ethanolic Extract 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^{c}	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}

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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]

Mean ± Std values with different superscript in the same row are significantly different (*P=.05*)

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

3.3 Anti-hyperglycemic Aqueous Fraction of *Balanites aegpytiaca* Stem-bark Ethanolic Extract in Streptozotocin Diabetic Rats

The effect of aqueous fraction of *Balanites aegyptiaca* stem-bark extract on blood glucose in STZ diabetic rats is given in Table 2. Diabetes is characterized with hyperglycemia, in the diabetic control rats the peak increase in fasting blood glucose level continued throughout the experimental period. However, decrease in fasting blood glucose level in Metformin and extract treated rats were significant (*P=.05*) compared to untreated diabetic.

3.4 Effect of Aqueous Fraction of *Balanites aegpytiaca* Stem-bark Ethanolic Extract 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 extract-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” and 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

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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 was a very sensitive signs of the glycolytic pathway and these are decreased in the liver of diabetic state [44]. The decrease of the activities of glycolytic enzymes in STZ-induced diabetic rats in our study was consistent with other studies on glycolytic enzymes [4,45]. However, administration of *Balanites aegyptiaca* stem-bark extract 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 [46].

	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]	209.0±08.2[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]

Table 2. Antihyperglycemic Effect of Oral Administration of Aqueous Fraction of *Balanites aegyptiaca* Stem-Bark Ethanolic Extract in Streptozotocin-induced Diabetic Rats

Mean ± Std values with different superscript in the same row are significantly different (*P=.05*)

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

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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±0.0.054^{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}

Mean ± Std values with different superscript in the same row are significantly different (*P=.05*)

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

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Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase [47]. 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 [48,49]. 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 [10,50,51]

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 [43,44]. The observed activation of glycogen synthase along with inhibition of phosphorylase, an enzyme that catalyzes glycogenolysis further supports insulinogenic activity of the plant. Gutierrez [52] has also reported this insulinogenic character of Prosthechea michuacana in STZ-induced diabetic rats.

Conclusion: It is concluded that aqueous fraction of *Balanites aegyptiaca* stem-bark ethanolic extract 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' were followed. All experiment have been examined and approved by the appropriate ethics committee.

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