Effect of Fractions of Balanites aegyptiaca Leaf Extracts On the Activities of Glucose Metabolizing Enzymes in Diabetic Rats

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Diabetes mellitus is a disorder associated with alteration in the activities of enzymes involved in glucose metabolism which disturbs maintenance of normal glycemia. Several plants extract were reported to have exerted significant regulatory effect on the activities of the enzymes. This study investigated effect of fractions of *Balanites aegyptiaca* leaves extract on the activities of key enzymes involved in glycolysis, gluconeogenesis, glycogenolysis and glycogenesis in diabetic rats. Fractions of plant leaves extract namely; diethyl ether, diethyl ether/chloroform, chloroform and methanol fractions were obtained via column fractionation of ethanol-aqueous extract of *Balanites aegyptiaca* leaves and administered separately to diabetic rats in group 1-4, group 5, 6 and 7 are positive, negative, and normal control rats groups. Enzymes activities were assayed from supernatant of liver homogenates. The study recorded a significant ($P<0.05$) reversal effect in the activities of glucose metabolic enzymes in diabetic treated rats in comparable to untreated diabetic rats. The fractions were able to enhanced the activities of glucokinase, phosphofructokinase, pyruvate kinase and glycogen synthase but depresses the activities of fructose-1,6-bisphosphatase, glucose-6-phosphatase phosphenol pyruvate carboxylkinase and glycogen phosphorylase. Liver glycogen and protein were elevated in the diabetic treated rats. The methanol fraction is identified to be the most potent. In conclusion, the study suggest fractions of *Balanites aegyptiaca* leaves extract exert glucose enzymes regulatory effect to combat diabetic mellitus in rats.

Keywords: *Effect, Fractions, Balanites aegyptiaca, Leaves extract, Glucose enzymes, Diabetic Rats*

1. Introduction

Diabetes mellitus is a metabolic disorder mainly distinguished by a very high level of glucose in the blood (hyperglycemia). Hyperglycemia largely results from hepatic glucose over production which occurs as a result of disturbance in the activities of enzymes involved in glycolysis, gluconeogenesis, glycogenesis and glycogenolysis [1]. Deficiency of insulin causes the suppression of glycolytic and glycogenic enzymes while promoting gluconeogenic and glycogenolytic enzymes activities [1,2], hence affecting glucose homeostasis [3,4].

Liver plays a major role in the glucose homeostasis by maintaining a balance between its utilization and production. Maintenance of normal glycemia requires matching of glucose utilization and endogenous production. This could be achieve via coordinated regulations of several metabolic pathways; glycolysis, gluconeogenesis, glycogenolysis, and glycogenesis [5,6]. Several regulatory enzymes like glucokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenol pruvate carboxlkinase, glycogen synthase and glycogen phosphorylase play key roles in these metabolic pathways [6]. Hence, any agent with potential to reverse hepatic carbohydrate metabolism might have influence on enzymes involved in glucose and glycogen metabolisms [7].

Agius [8] has reported that reversing the ac-

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tivities of enzymes involved in gluconeogenesis and glycogenolysis resulted in the lowering of fasting plasma glucose. Research on plants extract has shown significant impacts on the activities of enzymes involves in glucose metabolism in diabetes mellitus subjects [9,10].

The plant '*Balanites aegyptiaca*', also known as 'desert date' in English, a member of *Zygophyllaceae* family, is a common plant specie of the dry land areas of Africa and Asia [11,12]. In Nigeria, it is found mostly in the Northern region. It is known as '*Aduwa*' in Hausa, '*Utazi'* in Igbo, and '*Teji'* in Yoruba. *Balanites aegyptiaca* has a long history of traditional uses for wide ranges of disease [13].

Balanites aegyptiaca fruit and seed extracts has been reported as useful source of hypoglycemic remedy for the management of diabetes mellitus [14,15,16], and were postulated to have exerted antidiabetic effects via several mechanisms. The fruit extract was reported to have stimulated insulin secretion from the β-cell of the pancreas [14,17], inhibited intestinal α-amylase activity [18], and increased muscle basal glucose uptake [19]. While, the seed extract was reported to have exerted antihyperglycemic effect by ameliorating beta-cell dysfunction [16] and antioxidant activity [19].

In the studies reported by Chothani and Vaghasiya, and Abubakar *et al*., the leaves and stem-bark of *Balanites aegyptiaca* was reported to have been used by traditional medicine practitioners in the management of diabetes mellitus and various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever [13,20]. This claimed was confirmed by the authors' previous studies [21, 22]. Recent studies reported that *Balanites aegyptiaca* leaves extract stimulate erythrocytes glucose uptake in type II diabetic patients [23], and has the ability to inhibit alpha amylase activity *in vitro* [24].

However, no scientific study has been conducted to ascertain whether this plant part (leaves) could has effect on impaired activities of enzymes involved in glucose metabolism. Hence, this study investigated fractions of *Balanites aegyptiaca* leaves extract on the activities of enzymes catalyzes the rate limiting steps in glycolysis, gluconeogenesis, glycogenolysis and glycogenesis in diabetic rats.

2. Materials and Methods

2.1 Plant Collection and Identification

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

2.2 Chemicals

All solvents, chemicals and reagents used were of analytical grade and obtained from Sigma Aldrich, USA and BDH Ltd Poole, England. The solvents are; ethyl acetate, ethanol, diethyl ether, chloroform, and methanol. Chemicals include; Adenosine 5'-triphosphate, Glucose-6-phosphate, β-$NADP^{+}$, β-NADH, Glycogen, Fructose 6-phosphate, Phosphoenol pyruvate, Frutose-1,6-bisphosphate, D-Glucose, Adenosine 5-diphosphate (Sigma Aldrich, USA), Silica gel (60-200 mesh size), Metformin (Hovid, Malaysia) and Streptozotocin (Adooq Bioscience, LLC, United States). Enzymes include; Phosphoglucomutase, Glucose 6-phophate dehydrogenase, Pyruvate kinase, Lactate dehydrogenase (Sigma Aldrich, USA), Total protein assay reagent kit was from Agappe Diagnostics Switzerland GmbH.

2.3 Experimental Animals

A total of thirty-five (35) male wistar albino rats were used in the study. The rats were obtained from the Animal House, University of Jos, Plateau State, North Central of Nigeria and kept in clean cages with 12 hours / 12 hours light/dark photoperiod. Water and feed 'growers mash' (Vital feeds, Jos, Plateau State) were supplied *ad libitum*. The rats were allow to grow weighing between 180-230g before used. Experimental protocol was in conformity with national and international laws and guidelines for care and use of laboratory animals as in **'**Principle of Laboratory Animal Care [63].

2.4 Plant Leaves Extraction and Column Fractionation

The plant leaves after collection were air-dried under shade for a week period then pulverized using pestle and mortar into powdered form and then defatted with hexane as reported by Jung *et al* [25] followed by extraction as described by Govorko *et al* [26] with modification in choice of the extraction temperature (60 ^{o}C). Seven hundred and fifty gram (750g) of leaves powdered was defatted for 2 hours with 1200 ml hexane on mechanical shaker and then air-dried. Exact 200 g of defatted sample was mixed with 2000 ml of 80 % ethanol and heated to 60 °C for 2 hours and then kept at 20 ^{o}C for 10 hrs. It was filtered through a cheese cloth and the filtrate air-dried. The procedure was repeated twice with same amount of defatted sample. The ethanol leaves extract (183.44 g) was dissolved in water (500 ml) and partitioned with ethyl acetate (500 ml) at 20 ^{o}C for 2 hours then separated using a separating funnel (1000 ml). The aqueous fraction (ALF) was concentrated using a rotary evaporator at 40 ^{o}C and air dried.

The dried aqueous fraction (150 g) was subjected to column fractionation on silica gel (60-200 Mesh) packed in a chromatographic column and eluted with solvents of increasing polarity. Exact 5.0 g of ALF was dissolved in 5.0 ml of distilled water and then applied unto the chromatographic column and eluted with diethyl ether, diethyl ether/chloroform (1:1 v/v), chloroform and methanol respectively. Exact 400 ml of each solvent was poured into the column each time using Syringe. The eluted fractions were collected in aliquots of 40 ml in clean containers. The content in each container was allowed to evaporate at room temperature (25 ^{o}C) to solid form. The procedure was repeated until about 150 g of the ALF was used.

2.5 Study Design

The fractions of *Balanites aegyptiaca* leaves extract namely; diethyl ether (L1), diethyl ether/chloroform (L2), chloroform (L3) and methanol (L4) were administered separately to diabetic rats in groups (1-4) as follows: Group 1: received diethyl ether leaves fraction (L1), Group 2: received diethyl ether/ chloroform leaves fraction (L2), Group 3: received chloroform leaves fraction (L3), and Group 4: received methanol leaves fraction (L4). Whereas, Group 5 served as positive control and received Metformin (200 mg/kg body wt) [27], Group 6 served as negative control (not treated), and Group 7 was normal control. The plant leaves extract fractions were administered orally using oral gastric tube at a dose of 400 mg/kg body weight as determined by the authors' previous study [28]. Rats were sacrificed by decapitation at the end of the experiments, liver were collected, washed and homogenate where supernatant was used for assayed of the glucose metabolic enzymes activities.

2.6 Induction of Hyperglycemia

Hyperglycemia was induced in rats by intraperitoneal 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 then 1-week after 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 [29].

2.7 Assay of Hepatic Glucose Metabolic Enzymes

Glucokinase was assay following Goward's method [30]. Briefly, Exact 2.80 ml of the reaction cocktail (60 mM Tris HCl Buffer, , 20 mM $MgCl_2$, 110 mM KCl, 4 mM ATP, 12 mM D-glucose, G6PDH (10 U), 0.9 mM β-$NADP^+$) was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.10 ml enzyme (glucose-6-phosphate dehydrogenase) solution was added. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (60 mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then the absorbance was read at 340 nm after 10 minutes.

Calculation**:**

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

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where, 6.22 = millimolar extinction coeffi cient of NADPH at 340 nm,
3 = volume of mixture (ml),
0.1= Volume (in milliliters) of en zyme used,
df = dilution factor

Phosphofructokinase was assay by Hengartner and Harris method [31]. Exact 2.90 ml of the reaction cocktail (100 mM Tris-HCl buffer (pH 9.0 at 30 ^{o}C), 500 mM D- fructose-6-phosphate, 13.1 mM NADH and 10 mM $MgSO_4$, PK/LDH (5 U), 0.035 M ATP, 2.5 M KCl) was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (100 mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then incubated at 30 ^{o}C for 3 minutes and then the absorbance was read at 340 nm. Enzyme activity was determined using same formula as in Glucokinase.

Pyruvate kinase was assay by Pogson and Denton method [32]. Exact 2.89 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.10 ml enzyme (lactate dehydrogenase) solution was added. Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (72 mM Imidazole HCl) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^{o}C for 10 minutes then the absorbance was read at 340 nm. Enzyme activity was determined using same formula as in Glucokinase.

Phosphoenol pyruvate carboxylkinase was assay by Berndt and Ulbrich method [33]. Exact 2.90 ml of the reaction cocktail (100 µmoles Tris buffer (pH 8.0), 1 µmoles $MnCl_2$, 20 µmoles $MgSO_4$, 10 µmoles KCl, 10 µmoles KF, 7 µmoles ATP and 3 µmoles of oxaloacetate) was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (100 µmoles Tris HCl) was added to the blank test. The mixture was mixed and incubated at 37 ° C for 15 min., then 0.1 ml of TCA (3 M) was added to the content in both tubes. The mixture was centrifuged at 1000 x g for 10 min and 1.0 ml of 2,4-dinitrophenylhydrazine (0.3 % in 2 N HCl) was added to the supernatant. After about 20 min, 5.0 ml of ethyl acetate and water (1:1 v/v) was added. The ethyl acetate portion was decanted and 1.0 ml of the aqueous portion was neutralized with 0.10 ml of 3.5 N NaOH followed by the addition of 1.0 ml solution of 0.15 M NADH, pyruvate kinase (5 U) and lactic dehydrogenase (5 U). The mixture was incubated at 25 ^{o}C for 5 min then the absorbance was read at 340 nm. Enzyme activity was determined using same formula as in Glucokinase.

Glycogen phosphorylase was assay by Morgan and Parmeggiani method [34]. Exact 2.88 ml of the reaction cocktail (20 mM imidazole (pH 7, 30 ^{0}C), 0.01 % bovine serum albumin, 150 mM potassium acetate, 3 mM glycogen, 20 mM magnesium chloride, 15 mM cysteine, 10 mM inorganic phosphate, 0.1 mM ß-$NADP^{+}$, 20 µg glucose-6-phosphate dehydrogenase (10 U) 40 µg phosphoglucomutase) was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.01 ml each of glucose-6-phosphate dehydrogenase solution and phosphoglucomutase solution were added to the content in both tubes. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes.

Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (20 mM Imidazole) was added to the blank test. The mixture were mixed by inversion and was allow to stand for 10 minutes at 25 ^{o}C then the absorbance was read at 340 nm. Enzyme activity was determined using same formula as in Glucokinase.

Fructose-1,6-bisphosphatase was assay by Majumder and Eisenberg method [35]. Exact 2.90 ml of the reaction cocktail (100 mM Tris-HCl buffer (pH 8.0); 10 mM $MgCl_2$, 1 mM EDTA and 1 mM fructose-1, 6-bisphosphate) was pipetted into a clean test tubes labelled as sample test and blank test. Exact, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (100mM Tris HCl) was added to the blank test. The

mixture were mixed by inversion and incubated at 37 ^{o}C for 1 hour. Then, 1.0 ml of trichloroacetic acid (15 %) was added and centrifuged at 10,000 x g. Exact 1.5 ml of the supernatant was mixed with 1.5 ml of ammonium molybdate (2 g ammonium molybdate was dissolved in 5.55 ml conc. H_2SO_4 and was made to 100 ml with H_2O). After about 10 mins, absorbance of ammonium phosphomolybdate complex formed was read at 840 nm in a Spectrophotometer.

Calculations:

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

where, 3 = Total volume (in milliliters) of assay
0.3 = Millimolar extinction coefficient of inorganic phosphate at 840 nm

Glucose-6-phosphatase was assay by Baginski *et al* method [36]. Exact 2.90 ml of the reaction cocktail was pipetted into a clean test tubes labelled as sample test and blank test. Exactly, 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (135 mM Tris HCl) was added to the blank test. The mixture were mixed by inversion then incubated at 37 ^{o}C for 1 hour. Exactly 1.0 ml of trichloroacetic acid (15 %) was added and centrifuged at 10,000 x g. Exactly 1.5 ml of the supernatant was mixed with 1.5 ml of ammonium molybdate (2 g ammonium molybdate was dissolved in 5.55 ml conc. H_2SO_4 and was made to 100 ml with H_2O). The mixture were allow to stand for 10 minutes at 25 ^{o}C then the absorbance of ammonium phosphomolybdate complex formed was read at 840 nm in a Spectrophotometer. The enzyme activity was determined using same formula for Fructose-1,6 -bisphosphatase.

Glycogen synthase was assay by Danforth method [37]. Exactly 3.00 ml of the reaction cocktail (48 mM Tris-HCl, pH 8.2 at 30 ^{o}C, 12.4 mM $MgCl_2$, 1.0 mM EDTA, 1% glycogen, 3.63 mM UDP-glucose) was pipetted into a clean test tubes labelled as sample test and blank test. Then, exactly 0.10 ml sample (supernatant of the liver homogenate) was added to the content in the test tube labeled as sample test whereas 0.10.ml buffer (48 mM Tris HCl) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^{o}C for 5 minutes then the reaction was stoped by placing the tubes in boiling water bath. It was cooled with running tap water and centrifuged.

Phase II: Exactly 2.80 ml of the reaction cocktail Step II (48 mM Tris-HCl, pH 7.8, 25 mM potassium chloride, 1.0 mM EDTA, 60 mM $MgSO_4$, 5.2 mM PEP, PK/LDH (5 U), 6.6 mM NADH) was pipetted into a clean test tubes labelled as sample test and blank test. Then, 0.01 ml enzymes (pyruvate kinase and lactate dehydrogenase) solution was added. The mixture were mixed by inversion and then incubated at 30 ^{o}C for 5 minutes. Exactly, 0.10 ml sample (supernatant from the sample test reaction content in phase I) was added to the content in the test tube labeled as sample test whereas 0.10.ml of supernatant from the blank reaction content in phase I) was added to the blank test. The mixture was mixed by inversion and incubated at 30 ^{o}C for 10 minutes then the absorbance was read at 340 nm.

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

where, 2.91 = Total volume (in milliliters) of assay,
6.22 = mM Extinction Coeff of β-NAD^+ ,
0.1 = Volume (in milliliter) of enzyme,
5 = Time of Reaction (in mins) of Step 1.

2.8. Determinations of Liver Glycogen and Total Protein

The isolation and hydrolysis of glycogen in the liver tissues was done by the method described by Good *et al* [38]. While, glucose in the sample was measured by the method described by Carroll *et al* [39]. Briefly, A reagent blank was prepared by pipetting 1.0 ml of distilled water into a clean test tube. Exactly 1.0 ml of sample and standard glucose solution (0.5 mg/ml of glucose) was pipetted into a clean separate tubes. Then, 5.0 ml of anthrone reagent was delivered into each tube and tightly capped. The test tubes were placed in a cold water bath. After all tubes have reached the temperature of the cold water, they were immersed in a boiling water bath to a depth little above the level of the liquid in the tubes for 15 minutes and was then removed and placed in a cold water bath and cooled to room temperature.

Absorbance of test and standard were read

against the reagent blank at 620 nm. Glycogen was calculated using the formula: Glycogen (mg/g liver tissue) = ODu /ODs x 0.5 x Vol. of sample / g of sample x 100 x 0.9

where OD_U = optical density of the unknown,
OD_S = optical density of the standard,
0.5 =g of gluc in 0.5 ml of standard sol,
0.9 = factor for converting glucose value to glycogen value.

2. 9. 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) [40]. Significant difference was accepted at $P < 0.05$.

3. Results

3.1 Effect of Fractions of Balanites aegyptiaca Leaves Extract on Glycolytic Enzymes in Diabetic Rats

Effects of fractions of Balanites aegyptiaca leaves extract on key glycolytic enzymes: glucokinase (GK), phosphofructokinase (PFK), and pyruvate kinase (PK) activities in liver tissues of streptozotocin-induced diabetic rats is presented in Table 1. Significant changes ($P<0.05$) were observed in the activities of these enzymes. The fractions were able to enhance the activities of glycolytic enzymes; glucokinase (from 1.47±0.06 to 3.27±0.09 U/min/mg protein), phosphofructokinase (from 3.09±0.10 to 5.44±0.19 U/min/mg protein) and pyruvate kinase (from 0.96±0.16 to 2.19±0.13 U/min/mg protein) with methanol (L4) being the most potent in comparison to the diabetic rats groups that received L1, L2, & L3 fractions but less effective compared to metformin treated group.

3.2 Effect of Fractions of Balanites aegyptiaca Leaves Extract on Gluconeogenesis Enzymes in Diabetic Rats

The activities of glyconeogenic enzymes assayed in diabetic untreated and treated rats groups with metformin and fractions namely: diethyl ether (L1), diethyl ether/chloroform (L2), chloroform (L3) and methanol (L4) are presented in Table 2. The study recorded a significantly ($P<0.05$) depression in the activities of gluconeogenic enzymes; fructose-1,6-bisphosphatase (from 3.20±0.28 to 0.77±0.08 U/min/ µmole Pi liberated), glucose-6-phosphatase (from 1.90±0.15 to 0.24±0.04 U/min/µmole Pi

Table 1.Effect of Fractions of Balanites aegyptiaca Leaves Extract on Glycolytic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

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

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05)
L1 = Diethyl ether leaves fraction, L2 = Diethyl ether/chloroform leaves fraction, L3 =chloroform leaves fraction, L4 = Methanol leaves fraction

Table 2. Effect of Fractions of Balanites aegyptiaca Leaves Extract on Gluconeogenic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

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

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05)
L1 = Diethyl ether leaves fraction, L2 = Diethyl ether/chloroform leaves fraction, L3 =chloroform leaves fraction,
L4 = Methanol leaves fration

liberated) and phosphenolpyruvate carboxyl kinase (from 2.03±0.17 to 0.95±0.07 U/min/mg Protein) where methanol (L4) fraction was the most effective in comparison to the diabetic rats groups that received fractions; L1, L2, & L3 but less effective compared to metformin treated group.

3.3 Effect of Fractions of Balanites aegyptiaca Leaves Extract on Glycogen Metabolic Enzymes in Diabetic Rats

Activities of glycogen synthase (GS) and phosphorylase (GP) assayed in liver tissues of streptozotocin-induced diabetic rats groups received diethyl ether, diethyl ether/chloroform, chloroform and methanol fractions of Balanites aegyptiaca leaves

Table 3. Effect of Fractions of Balanites aegyptiaca Leaves Extract on Glycogen Metabolic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

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

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05)
L1 = Diethyl ether leaves fraction, L2 = Diethyl ether/chloroform leaves fraction, L3 =chloroform leaves fraction,
L4 = Methanol leaves fraction

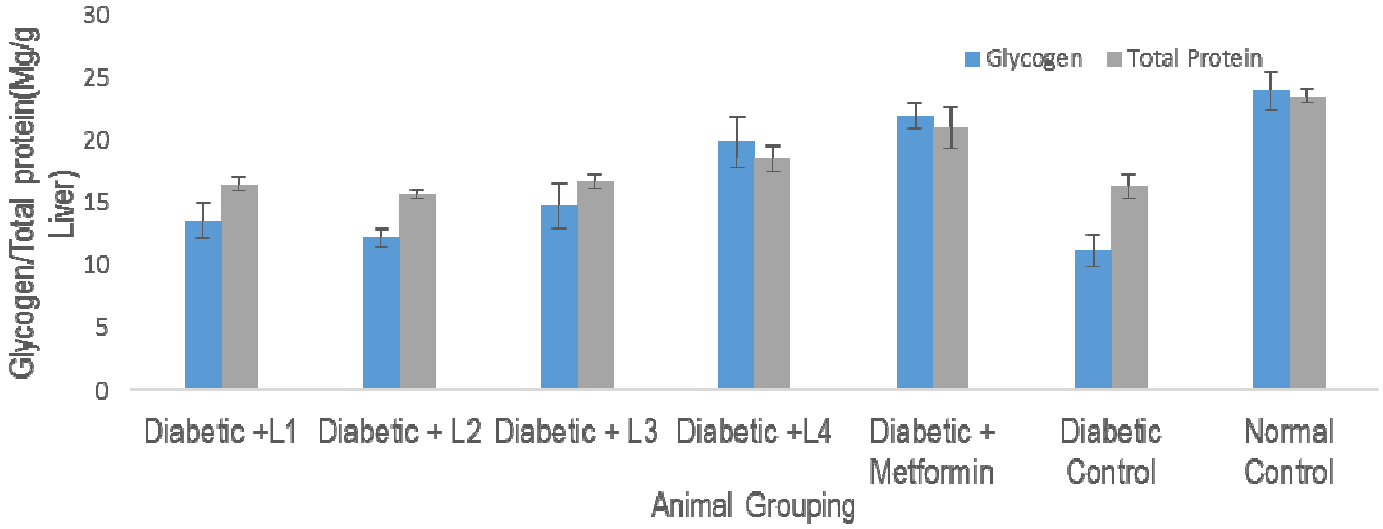

Figure I. Effect of Fractions of Balanites aegyptiaca Leaves Extract on Liver Glycogen and Total Protein of Streptozotocin-induced Diabetic Rats

L1 = diethyl ether fraction, L2 = diethyl ether/chloroform fraction, L3 = chloroform fraction, L4= methanol fraction

extract is presented in Table 3. Phosphorylase activity was significantly ($P<0.05$) suppressed (from 2.56±0.27 to 1.02±0.29 U/min/mg protein) whereas glycogen synthase was activated (from 0.10±0.00 to 0.28±0.04 U/min/mg protein) as recorded in diabetic rats' groups treated with fractions. Methanol fraction (L4) was significantly ($P<0.05$) compared to the effects of the other fractions: diethyl ether (L1), diethyl ether/chloroform (L2), and chloroform (L3).

3.4 Effect of Fractions of Balanites aegpytiaca Leaves Extract on Liver Glycogen and Total Protein in Diabetic Rats

There was a significant reduction ($P<0.05$) in glycogen content of diabetic untreated rats (10.69±0.32 mg/g liver) in comparison to the diabetic treated rats with diethyl ether (L1), diethyl ether/chloroform (L2), chloroform (L3) and methanol (L4) fractions (Figure I). Significant elevation ($P<0.05$) in glycogen content was observed in diabetic rats that received L4 fraction (19.90±2.07 mg/g liver) and metformin (22.00±1.05 mg/g liver) in comparison to diabetic untreated rats (11.20±1.24 mg/g Liver). A significant ($P<0.05$) change was recorded in liver total protein of diabetic rats' groups treated with fractions and metformin in a comparable manner to diabetic untreated rats (Figure I). The methanol (L4) fraction seem to be more effective by recording (18.55±1.05 mg/g Liver), metformin (21.03±1.71 mg/g Liver) in comparison with other fractions and diabetic untreated rats group (16.29±0.96 mg/g Liver).

4. Discussion

Liver is an insulin-sensitive tissue and plays a major role in glucose metabolism by regulating the interaction between glucose utilization and production [41]. A partial or total deficiency of insulin causes derangement in glucose metabolism that decreases the activities of glycolytic enzymes causing impaired peripheral glucose utilization while promoting gluconeogenesis [42]. This study determine whether fractions of *Balanites aegyptiaca* leaves extract modulates enzymes involved in glucose and glycogen metabolism.

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 [43]. Reduced activities of these enzymes in this study are consistent with other studies on glycolytic enzymes

[1, 44]. Insulin influences the intracellular utilization of glucose by promoting glycolysis by increasing the activity and amount of glycolytic enzymes [44,45]. Administration of fractions of *Balanites aegyptiaca* leaves extract 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 [46, 47].

Specifically, the activity of glucokinase (GK), a rate limiting enzyme involved in the hepatic storage and utilization of glucose showed a significant change in activity in this study as reported by Smith *et al* [48] and Agius [8]. The activity of hepatic GK is reported to be reduced in diabetes mellitus and can be activated by an activator [49]. Plant extracts have been reported to exert antihyperglycemic activity in part by promoting the activity of this enzyme [50,51]. Glucokinase activity in diabetic rats treated with fractions of *Balanites aegyptiaca* leaves extract might have improve glycolysis. Shafik *et al* [19] have shown that extract of *Balanites aegyptiaca* seed-kernel promotes the activity of hexokinase, suggesting glycolysis stimulatory effect of the plant.

This study also notice that phosphofructokinase was enhanced significantly. It was reported that the first irreversible reaction unique to glycolytic pathway is the phosphorylation of fructose 6-phosphate to fructose-1,6-bisphosphate which is catalyzed by the phosphofructokinase [52, 53]. Increased phosphofructokinase activity in the diabetic rats treated with fractions of *Balanites aegyptiaca* leaves extract particularly the methanol (L4) fraction suggests that it contain compounds that regulate glycolysis.

Gluconeogenesis is a main cause of the elevated hepatic glucose production contributing 50-60 % of the released glucose [54]. Insulin decreases gluconeogenesis by decreasing the activities of enzymes, such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase [2, 55]. The rate of gluconeogenesis is regulated mainly by the activity of two rate limiting gluconeogenic enzymes; glucose-6-phosphatase and fructose-1,6-bisphosphatase. G6pase, is a key enzymes in the homeostatic regulation of blood glucose and is critical in providing glucose to other organs during diabetes mellitus [6]. Whereas, Fructose-1,6-Bisphosphatase is key regulator enzymes of the hepatic gluconeogenesis and appears as target for efficient and safe glycemic control in diabetes mellitus [56].

This study recorded an enhanced activities of gluconeogenic enzymes in diabetic untreated rats compared to the treated diabetic rats. It suggest that the fractions of *Balanites aegyptiaca* leaves extract contain components that has the ability to suppress the activities of these enzymes. This findings is in line with other studies where several plants extract were reported to have suppressed the activities of these enzymes in diabetic animals [27,57, 58]**.** Shafik *et al* [19] have reported that extract of *Balanites aegyptiaca* seed-kernel suppressed glucose -6-phosphatase activity.

Glycogen synthase (GS) catalyzes the rate limiting step in glycogen synthesis and is thus responsible for the storage of glucose as glycogen in the liver. Fractions of *Balanites aegyptiaca* leaves extract appear to have effect on both glycogen synthase activity and phosphorylase by enhancing glycogen synthase but suppressed phosphorylase activity in the diabetic treated rats. This is supported by the relative change in glycogen content in liver of the diabetic treated rats. Some plants extract have been reported to regulate glycogen enzymes leading to increased hepatic glycogen content [59, 60]. According to Gutierrez [61], activation of glycogen synthase by plant suggested insulinogenic character; going by this statement one may propose that fractions of *Balanites aegyptiaca* leaves extract particularly the methanol (L4) fraction contains component that exhibits insulin like effect.

It has been reported that glycogen levels in liver tissues decreases as the influx of glucose in to the liver is inhibited in the absence of insulin and recovers on insulin treatment [62]. Data from this study showed significant reduction in glycogen content in liver of untreated diabetic rats; decreased glycogen content may result from insulin deficiency leading to a decreased influx of glucose in liver and/ or decreased activity of glycogen synthase in the liver. Increased glycogen content in rats that received fractions of *Balanites aegyptiaca* leaves ex-

tract might probably be by direct effect on the enzymes (glycogen synthase) resulting in its reactivation. Increased hepatic glycogen content due to activation of glycogen synthase by plants extract in diabetic rats has been reported by Jang *et al* [59] and Ramanchadran and Saravanan [60].

5. Conclusion

In conclusion, the study shows fractions of *Balanites aegyptiaca* leaves extract are able to reverse glucose metabolizing enzymes activities altered in the diabetic rats where, methanol fraction (L4) is identified as the most potent. Investigation into the molecular mechanism to further ascertain the effect of the plant's leaves extract on glucose metabolizing enzymes is recommended.

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Conflict of interest

The authors declare that there is no conflict of interest regarding this article.

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