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Research Article
Fractionated Ethanol Extract of *Balanites aegyptiaca* Fruit-Mesocarp Effect on Diabetes Mellitus Model Rats

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Abstract

Background and Objective: *Balanites aegyptiaca* fruit is a useful source of hypoglycaemic remedy for management of diabetes in some part of Northern Nigeria. This study evaluated the effects of fractionated ethanol extracts of *Balanites aegyptiaca* fruit-mesocarp (BAFM) in streptozotocin-induced diabetic rats. **Materials and Methods:** Ethanol crude extract of BAFM was fractionated with water and ethyl acetate (1:1 v/v) then separated to obtain the specific extract-fraction. The extract-fractions were evaluated for antidiabetic activities in rats induced with diabetes. Diabetes mellitus was induced in male Wistar rats by intra-peritoneal injection of streptozotocin at a dose of 60 mg kg^{-1} body weight. **Results:** From the results, rats induced with diabetes were characterized by low serum insulin, hyperglycemia and decrease in body weights. Treatment with the extract-fractions of BAFM elevated serum insulin, lowered fasting blood glucose levels and reversed serum total cholesterol, triglyceride, low density lipoprotein cholesterol and very low density lipoprotein cholesterol levels, serum albumin and total protein. The aqueous fraction (AFF) was more comparable in lowering fasting blood glucose by 18.61% when compared to 24.62% by metformin. A significant increase in serum insulin levels was recorded in diabetic rats treated with AFF when compared to diabetic control rats and diabetic rats treated with ethyl acetate fraction. **Conclusion:** Both aqueous and ethyl acetate extract-fractions of BAFM exert hypoglycaemic, antihyperglycaemic and antilipidaemic effects. However, the aqueous fraction was more potent. This study is a step towards the isolation of bioactive components. Further research is needed to explore the bioactive component(s) responsible for the antidiabetic effects.

Key words: Diabetes, *Balanites aegyptiaca*, fruit-mesocarp, extract-fractions, model rats

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM), commonly called diabetes is a disease in which the treatment is still a challenge globally and stable treatment is yet to be achieved. It is a disorder of carbohydrate metabolism characterized by a chronic hyperglycemia due to low plasma insulin level or insensitivity of target organs to insulin. Diabetes is one of the key health problems affecting millions of people worldwide not minding the age, sex and socioeconomic status[1-3]. There are several types of diabetes but the two most common forms are type-1 and type-2 diabetes. The cause of type-1 diabetes is unknown and is currently not preventable[1]. However, it is believed to be the consequence of an auto-immune response leading to a breakdown of insulin producing cells[4]. The pathogenesis of type-2 diabetes differs from type-1 diabetes in 2 key features namely, insulin resistance and β-cell failure[5].

Evaluation of medicinal plants for the management of DM is highly recommended considering the prevalence and complication of and death rate caused by the disease. Research on plants like *Galega officinalis* has yielded positive results by identifying metformin as the bioactive antidiabetic compound in the plant[6]. Metformin is one of the antidiabetic drugs available for the treatment of diabetes mellitus. Emphasis of research has been on utilizing medicinal plants that have long and proven history of curing or treating various ailments[7]. One of such medicinal plant is *Balanites aegyptiaca*.

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[8]. 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[9]. Scientific studies have reported potential antidiabetic activity of extracts from *Balanites aegyptiaca* parts[10-13]. Zaahkouk *et al.*[14] reported potential hypoglycaemic activities on *Balanites aegyptiaca* fruit pericarp. On the bases of these reports, the main purpose of this study was focused on the identification of the nature of the bioactive components present in the plant fruit-mesocarp. This was achieved by evaluation of the antihyperglycemic and antilipidemic potential of fractionated ethanol extracts, aqueous fraction and ethyl acetate fraction of *Balanites aegyptiaca* fruit-mesocarp (BAFM) in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant sample collection and identification: The fruit-mesocarp of *Balanites aegyptiaca* were collected from a village called Gubi in Bauchi LGA, Bauchi State, Nigeria (latitude 10° 45' N and longitude 9° 82' E). The identification and authentication (voucher specimen number 900175) was performed by the Herbarium Unit of the Biological Science Department, Ahmadu Bello University Zaria, Nigeria. This study was conducted between August, 2017 and February, 2018.

Experimental animals: Albino Wistar rats (25) used in the study were purchased from Animal House, University of Jos, Plateau State, Nigeria. Before the commencement of the study, the rats were allowed to acclimatize for 2 weeks under standard environmental conditions in clean cages with normal day light/dark period. During this period they were provided with water and feed 'growers mash' (Vital feeds, Jos) *ad libitum*. Before starting the experiment, only rats that had attained a body weight between 180-230 g were included in the study. The protocol used in the study complied with national and international laws and guidelines for the care and use of laboratory animals as in 'principle of laboratory animal care'[15].

Chemicals/reagents: Analytical grade of chemicals and reagents were used in the study. They were procured from Agape Diagnostics, Switzerland, British Drug House, England, Randox Laboratory, UK and Sigma Aldrich, USA.

Preparation of plant sample

Defattening, extraction and fractionation: After collection of the BAFM, they were air-dried in shade for a period of 1 week and then pulverized using pestle and mortar in powder form. The pulverized samples were defatted, while the extraction and fractionation was performed with modification in the choice of the extraction temperature (60°C). Seven hundred and fifty gram (750 g) powdered plant fruit-mesocarp was defatted for 2 h with 1200 mL hexane on mechanical shaker[16-18].

The hexane solvent was discarded and the defatted samples were air-dried. Thereafter, 200 g of the defatted fruit-mesocarp was mixed with 2000 mL of 80% ethanol and heated to 60°C for 2 h. The extraction was continued for an additional 10 h at 20°C. The mixture was filtered through a cheese cloth and resulting ethanol extract was air-dried. The ethanol crude extract of fruit-mesocarp obtained was fractionated by dissolution in water (500 mL) and partitioned

with ethyl acetate (500 mL) at 20°C for 2 h, then separated using a separating funnel (1000 mL) to obtain the aqueous and ethyl acetate fractions, respectively. The fractions (extract-fractions) were concentrated using a rotary evaporator at 40°C and air dried. The dried extract-fractions of BAFM were stored in air-tight containers and kept in a refrigerator at 4°C until used.

Phytochemical analysis: Phytochemical analysis of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp was carried out following the procedures described by Harborne[19].

Induction of diabetes mellitus: Type-1 diabetes mellitus was induced in rats by intra-peritoneal injection of streptozotocin (STZ) at a dose of 60 mg kg^{-1} body weight dissolved in 0.1 M citrate buffer (pH 4.5). Rats were supplied with 10 % glucose solution in their drinking water for 48 h after STZ injection in order to prevent severe hypoglycaemia. After 72 h, blood glucose levels were checked and subsequent 1 week intervals to identify the onset and continued presence of hyperglycemia[20]. Rats with fasting blood glucose levels $\geq$200 mg dL^{-1} were considered diabetic and selected for the study[21].

Grouping of animals and treatment: The fractionated ethanol extracts of BAFM were evaluated for antidiabetic activity in streptozotocin-induced diabetic rats' groups as follows:

- **Group A (Diabetic+AFF):** Diabetic rats treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract
- **Group B (Diabetic+EFF):** Diabetic rats treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract
- **Group C (Diabetic+Metformin):** Diabetic rats treated with metformin at 200 mg kg^{-1} body weight[22]
- **Group D (Diabetic control):** Diabetic rats without treatment
- **Group E (Normal control):** Healthy rats without diabetes

The fractionated extracts (extract-fractions) were administered orally using oral gastric tube. Exactly, 400 mg kg^{-1} body weight of plant extract-fractions were administered to various diabetic rats' groups. This dose used in the treatment was derived from our previous acute toxicity report on the ethanol extract-fractions of BAFM[23].

Feed and fluid intake estimations: During the experiment, feed and fluid intake was recorded daily. The feed was weighed using a weighing scale to ascertain the quantity given and the remnants in each rat groups after 24 h. The volume of fluid provided before and after 24 h was measured using a measuring cylinder (1000 mL).

Animal body weight and pack cell volume estimations: Animal weight was determined weekly by weighing the rats using a weighing scale. The rats were weighed after an overnight fast by properly placing the rats in the weighing pan of the weighing scale thereafter the weights was recorded. The percentage pack cell volume (PCV) of rats was determined by the method described by Schalm *et al.*[24], using a micro-hematocrit.

The experimental duration for this study was 28 days and on day 29th, animals were anaesthetized with chloroform and then sacrificed. Blood was collected and centrifuge at 3000 rpm for 15 min to obtain serum which was separated and stored at -30°C for the determinations of biochemical parameters.

Biochemical parameters determinations: The serum biochemical parameters namely, total protein, albumin, triglyceride, total cholesterol, high density lipoprotein were assay using kits from Agape Diagnostics Switzerland GmbH and Randox Lab. UK. The procedures were followed according to the manufacturer instruction.

Determination of blood glucose level: The determination of blood glucose was done as described by Beach and Turner[25]. After an overnight fast, blood was collected from the tail vein of rats and was used to measure their blood glucose levels using a glucometer.

The total area under fasting blood glucose curve was determined using the formula described by Tai[25]:

$$\text{Area} = \tfrac{1}{2} \sum \text{Xi}_{-1} (Y_{i-1}+Y_i)$$

$$i = 1 \text{ (Tai formula)}$$

Determination of total protein: The determination of total protein was done according to the method of Bradford[26]. The protein was measured spectrophotometrically at 595 nm.

Determination of serum albumin: The determination of serum albumin was done according to the method described by Doumas *et al.*[27]. The albumin-BCG complex maximally absorbs at 630 nm. The absorbance is directly proportional to the concentration of the albumin in the sample.

Determination of serum triglyceride: The determination of serum triglyceride (TG) was done according to the method described by Fossati and Prencipe[28]. The H_2O_2 reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to produce red chromogen which was measured spectrophotometrically at 546 nm.

Determination total cholesterol: The method described by Roeschlau *et al.*[29], was employed in the determination of total cholesterol (TC).

Determination of high density lipoprotein cholesterol: The level of high density lipoprotein cholesterol (HDL-C) was determined using the method described by Lopes-Virella *et al.*[30]. After centrifugation, high density lipoprotein (HDL) was obtained as the supernatant. The HDL content of the supernatant was measured via an enzymatic method.

LDL cholesterol and VLDL cholesterol: LDL and VLDL were determined using the following formulas below[31]:

$$\text{LDL} - \text{Cholesterol concentration (mg dL}^{-1}\text{)} = \text{TC-HDL-C+}\left(\text{Triglycerides}/5\right)$$

$$\text{VLDL} - \text{Cholesterol concentration (mg dL}^{-1}\text{)} = \text{Triglycerides}/5$$

Determination of alanine and aspartate amino transaminase: The assay of aspartate and alanine aminotransferases activity was done by the method of Reitman and Frankel using assay kits[32].

Determination of fructosamine: Serum fructosamine was determined as described by Acharya and Manning[33]. In the procedure 0.2 mL of serum and standard (dihydroxyacetone prepared in varied concentrations, 0.03, 0.06, 0.12, 0.23, 0.46, 0.92 mmol L^{-1}) was mixed with 1.0 mL of 9 g L^{-1} NaCl and incubated at 37°C for 10 min. Then, 1.0 mL phenylhydrazine was added, the absorbance was read at 350 nm after 10 min. The standard curve was plotted using the absorbance values against concentrations of dihydroxyacetone. The unknown serum fructosamine concentration (mmol L^{-1}) was extrapolated from the curve.

Determination of serum insulin level: Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) method as described by Clark and Hales[34]. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and antibodies bound to the micro titration 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 a micro-plate auto reader at 450 nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

Percentage change and available serum insulin: Determination of percentage available serum insulin and percentage change in available serum insulin was calculated according to the procedure described by Okoduwa *et al.*[35].

$$\text{Change in serum insulin (\%)} = \frac{\text{Concentration of serum insulin in expt.} - \text{Concentration of insulin in NC}}{\text{Concentration of insulin in NC}} \times 100$$

$$\text{Available serum insulin (\%)} = \frac{\text{Concentration of serum insulin in expt.} - \text{Concentration of insulin in NC}}{\text{Concentration of insulin in NC}} \times 100$$

Where:

Expt. = Experimental test group
NC = Normal control group

Statistical analysis: All statistical analysis was conducted using the computer software, statistical package for the social sciences (SPSS Cary, NC, USA). The results of the experiments were pooled and expressed as mean±standard deviation (SD). The means were analyzed by one-way analysis of variance (ANOVA) and *post hoc* test. Differences between extract-fractions and animal groups were compared using Duncan's multiple range test (DMRT)[36]. The value of $p<0.05$ was considered statistically significant.

RESULTS

Yield and phytochemical constituents of extract-fractions: The yield of the extract-fractions namely, aqueous fruit-mesocarp fraction (AFF) and ethyl acetate fruit-mesocarp fraction (EFF) is presented in Table 1. The results of the phytochemical screening of AFF and EFF are also presented in Table 1.

Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on fluid and feed intake: The feed and fluid intake of STZ-induced diabetic treated, diabetic untreated and non-diabetic rats groups are presented in Table 2. No significant ($p>0.05$) changes were observed in the feed or fluid intake among the STZ-induced diabetic rats treated with

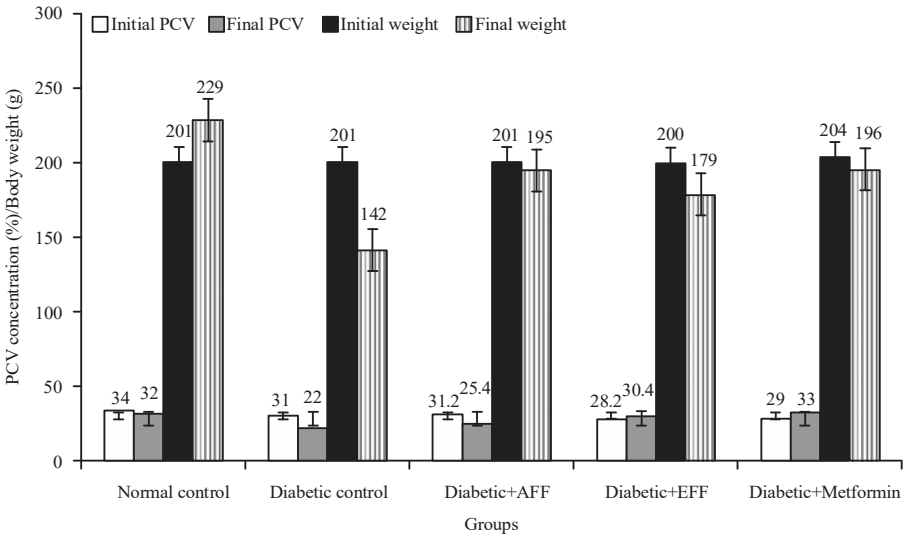

Fig. 1: Effect of oral administration of ethanol extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on body weight and packed cell volume (PCV) of diabetic and non diabetic rats

Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.w., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes, PCV: Packed cell volume

Table 1: Yield and phytochemical constituents of ethanol extract-fractions of *Balanites aegyptiaca* fruit-mesocarp

Yield and phytoconstituents	Aqueous fruit-mesocarp fraction (AFF)	Ethyl acetate fruit-mesocarp fraction (EFF)
Yield (g/750 g)	130.80	18.96
Saponins	+++	++
Terpenoids	-	-
Flavonoids	++	+
Phenolics	+	++
Tannins	++	++

+++: Present in high concentration, ++: Moderately present, +: Present in low concentration, -: Absent or not detected

Table 2: Effect of oral administration of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on feed and fluid intake of diabetic and non diabetic rats

	Animal groups				
Parameters	Normal control	Diabetic control	Diabetic+AFF	Diabetic+EFF	Diabetic+Metformin
Feed (g/day/rat)	23.27±6.51^{a}	32.37±3.63^{bc}	27.67±2.54^{b}	27.99±2.97^{b}	25.37±3.50^{b}
Fluid (mL/day/rat)	17.67±2.19^{a}	29.04±9.15^{bc}	26.35±7.40^{b}	25.59±6.62^{b}	21.47±4.91^{b}

Values are mean±SD of 28 determinants, values with different superscripts across the rows are significantly different ($p<0.05$), Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes

extract-fractions and metformin but a significant ($p<0.05$) difference were seen when compared to diabetic and normal control rats groups.

Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on body weight and pack cell volume (PCV): Change in body weight and PCV of STZ-induced diabetic rats following treatment with ethyl acetate and aqueous fractions of *Balanites aegyptiaca* fruit-mesocarp is presented in Fig. 1. Body weight of the diabetic treated and untreated rats in general were observed to have decreased after 7 days. The decrease in body weight continued throughout the experimental period in the untreated diabetic rats. As treatment advances among the diabetic treated rats, again in body weight was recorded. Fluctuation of PCV in diabetic rats groups treated with the ethanol extract-fractions was observed. However, the levels were still within the normal range as compared with the normal control rats.

Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on fasting blood glucose: The effect of ethyl acetate and aqueous fractions of the fractionated ethanol

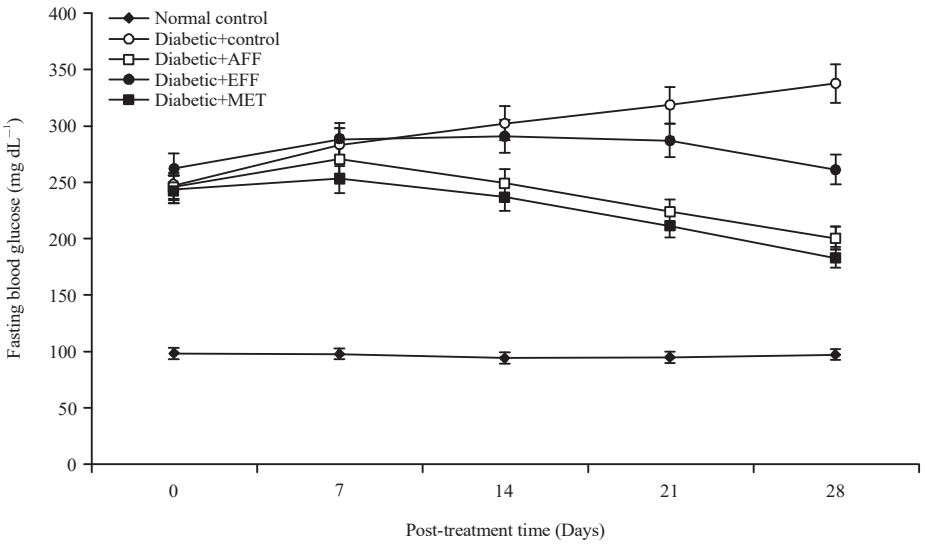

Fig. 2: Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on fasting blood glucose in diabetic and non diabetic rats

Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes

Table 3: Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on status of serum insulin concentration in diabetic and non diabetic rats

Groups	Serum insulin (ng L^{-1})	Change in serum insulin (%)	Available serum insulin (%)
Normal control	0.45	0	100.00
Diabetic control	0.09	-77.80	22.20
Diabetic+AFF	0.33	-26.67	73.33
Diabetic+EFF	0.13	-71.11	28.89
Diabetic+Metformin	0.19	-57.78	42.22

Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes

extract of BAFM on blood glucose level in STZ diabetic rats is given in Fig. 2. A gradual raised in fasting blood glucose was recorded from diabetic control animals and continued throughout the experimental period (246.80±7.46 to 336.69±11.91 mg dL^{-1}). Decrease in fasting blood glucose levels among the diabetic rats that were treated with metformin and plant extract-fractions was time-dependent and significantly different ($p<0.05$) when compared among the extract-fraction treated groups and the diabetic untreated. Diabetic animals treated with metformin had a reduction in fasting blood glucose by 24.62% followed by the group that received aqueous fruit-mesocarp fraction (AFF) 18.61%. The aqueous fruit-mesocarp fraction was more active when compared to the ethyl acetate fraction.

Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on insulin serum level: Increased in serum insulin levels were recorded following oral administration of the extract-fractions of BAFM to diabetic rats (Fig. 3). Serum insulin levels of diabetic control rats (0.09±0.02 ng L^{-1}) were significantly different ($p<0.05$) from diabetic rats treated with aqueous fruit-mesocarp fraction (0.33±0.10 ng L^{-1}) and ethyl acetate fruit-mesocarp fraction (0.13±0.02 ng L^{-1}). A severe decrease in serum insulin was observed among the diabetic control (-77.8%) when compared to the normal control. The available serum insulin in the diabetic control group was 22.2% but after treatment with the extract-fractions, significant improvement in the concentration levels of serum insulin was observed (Table 3).

Antihyperlipidemic effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp: The diabetes induced rats showed a significant increase in cholesterol, TG, VLDL and decrease in HDL levels compared to the normal control and diabetic treated rats. Administration of extract-fractions of *Balanites aegyptiaca* leaf to diabetic rats significantly

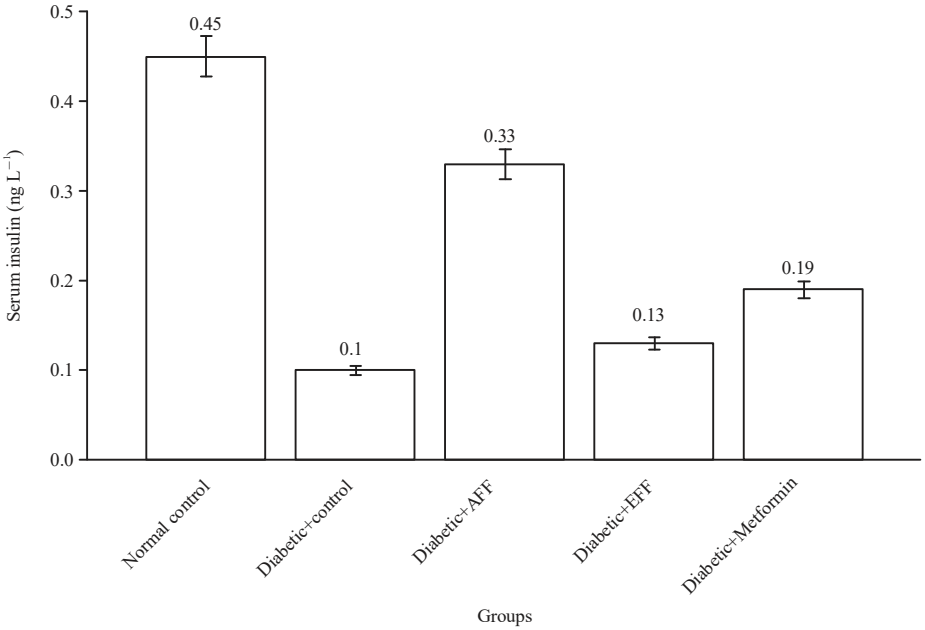

Fig. 3: Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on serum insulin level in diabetic and non diabetic rats

Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes

Table 4: Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on lipid profile in diabetic and non diabetic rats

	Groups				
Parameters (mg dL^{-1})	Normal control	Diabetic control	Diabetic+AFF	Diabetic+EFF	Diabetic+Metformin
Cholesterol	76.31±3.19^{a}	232.00±2.96^{bcde}	170.46±2.96^{b}	217.85±3.54^{bcd}	180.62±3.19^{bc}
Triglyceride	97.82±2.90^{a}	207.13±6.05^{bc}	140.44±1.71^{b}	143.13±3.46^{b}	99.91±5.55^{a}
HDL-cholesterol	47.78±3.01^{b}	27.22±5.07^{a}	50.83±0.95^{bc}	55.49±7.90^{bcd}	66.35±5.53^{bcde}
LDL-cholesterol	47.19±3.56^{a}	185.13±4.78^{bcd}	132.21±3.20^{b}	178.12±3.71^{bcd}	147.36±4.57^{bc}
VLDL	19.57±0.58^{a}	41.43±1.21^{bc}	28.09±0.34^{b}	28.63±0.69^{b}	19.98±1.11^{a}

Values are mean±SD of 5 determinants, values with different superscripts across the rows are significantly different ($p<0.05$), Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes

conserves lipids profile levels (Table 4). Serum cholesterol level in diabetic rats (232.00±2.96 mg dL^{-1}) was significantly different ($p<0.05$) compared to values obtained from diabetic rats treated with metformin (180.61±3.19 mg dL^{-1}) and aqueous fruit-mesocarp fraction (170.46±2.96 mg dL^{-1}). Similarly, elevated serum triglyceride levels recorded in the diabetic untreated rats (207.13±6.05 mg dL^{-1}) was significantly ($p<0.05$) lower in the diabetic rats treated with aqueous fruit-mesocarp fraction (140.44±1.71 mg dL^{-1}). Also, low levels of HDL-cholesterol was recorded in diabetic untreated rats (27.22±5.07 mg dL^{-1}) but elevated level was recorded in diabetic rats treated with aqueous fruit-mesocarp fraction (50.83±0.95 mg dL^{-1}).

Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on liver parameters: Hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, serum albumin and total protein of diabetic untreated and diabetic treated rats with extract-fractions of BAFM are presented in Table 5. There was a significant alternation ($p<0.05$) in the activities of both aspartate (AST) and alanine (ALT) aminotransferases in diabetic rats that received plant extract-fractions. The activities of AST were declined significantly whereas that of the ALT elevated in comparison to the control rats' groups. In contrast, there was a significant decrease ($p<0.05$) in the activities of the enzymes in animals treated with metformin in comparison to the

Table 5: Effect of extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on liver parameters in diabetic and non diabetic rats

	Groups				
Parameters	Normal control	Diabetic control	Diabetic+AFF	Diabetic+EFF	Diabetic+Metformin
Albumin (g dL^{-1})	4.28 ± 0.01^{bcdef}	2.21 ± 0.16^{a}	3.94 ± 0.14^{bcde}	4.24 ± 0.01^{bcdef}	3.59 ± 0.00^{bcd}
Total protein (mg g^{-1} liver)	26.95 ± 0.04^{bcdef}	17.35 ± 1.96^{a}	19.63 ± 0.02^{b}	21.06 ± 0.06^{bcd}	25.01 ± 0.06^{bcde}
Fructosamine (mmol L^{-1})$\times10^{-1}$	0.30 ± 0.16^{a}	2.91 ± 0.70^{bcd}	0.67 ± 0.01^{bc}	0.56 ± 0.12^{bc}	0.48 ± 0.01^{b}
ALT (U L^{-1})	3.48 ± 0.11^{a}	4.28 ± 0.23^{bc}	6.08 ± 0.18^{bcde}	5.96 ± 0.78^{bcde}	3.24 ± 0.82^{a}
AST (U L^{-1})	11.70 ± 0.27^{bc}	13.30 ± 0.27^{bcde}	11.50 ± 0.35^{b}	9.60 ± 1.67^{a}	12.90 ± 0.65^{bcd}

Values are mean±SD of 5 determinants, values with different superscripts across the rows are significantly different ($p<0.05$), Diabetic+AFF: Diabetic rats group treated with aqueous fruit-mesocarp fraction (AFF) of the fractionated ethanol crude extract, Diabetic+EFF: Diabetic rats group treated with ethyl acetate fruit-mesocarp fraction (EFF) of the fractionated ethanol crude extract, Diabetic+Metformin: Diabetic rats group treated with standard drug (metformin) at 200 mg kg^{-1} b.wt., Diabetic control: Diabetic rats group without treatment, Normal control: Normal healthy rats group without diabetes, ALT: Alanine transaminase, AST: Aspartate transaminase

normal and diabetic control rats. There was significant ($p<0.05$) decrease in the concentration of albumin (ALB) of untreated diabetic rats (2.21±0.16 g dL^{-1}) compared to all the diabetic treated groups.

DISCUSSION

The findings from the study showed low serum insulin but elevated fasting blood glucose and altered lipid profile in the diabetic rats groups. In a comparison of activity between the aqueous fraction and ethyl acetate fraction, the study revealed that the aqueous fraction was significantly highly effective compared to the ethyl acetate fraction. By implications, it signify that the aqueous fraction contains molecules or components that are hypoglycaemic. The hypoglycaemic activity of molecules in the aqueous fraction may likely be a reflection of their regulatory effect on the activities of glucose metabolizing enzymes as reported by Mhya *et al.*[37].

The increased feed and fluid intake by diabetic rats may be due to chronic reduction in glucose utilization by the cells and considerable loss of glucose in the urine. It has been reported that failure of the kidney to reabsorb glucose in diabetic state results in severe loss of renal glucose[38]. Loss of renal glucose is accompanied by loss of water and some electrolytes. Administration of *Balanites aegyptiaca* fruit-mesocarp extract-fractions may have improved glycaemic control which prevented excess food and fluid intake in diabetic treated rats. Srinivasan *et al.*[39] had reported that eugenol, a phenolic compound argument the desire for food and fluid intake under diabetic condition via glycemic control. The results obtained from this study showed that fractions of fractionated ethanol extract of BAFM, particularly the aqueous fruit-mesocarp fraction exhibited hypoglycaemic effect in diabetic rats. The blood glucose lowering capability of the fractions of fractionated ethanol extract of BAFM may be due to the presence of compounds such as saponins, rutin and vanillic and syringic acids that are known to exhibit hypoglycaemic activity and have been reported to be present in the fruit extract[11-13].

Several postulations have been made to explain how plant extracts lowered blood glucose, among was the reports by Zaahkouk *et al.*[14] that *Balanites aegyptiaca* stimulates insulin secretion while Gad *et al.*[40] suggested that inhibition of intestinal α-amylase activity. Motaal *et al.*[11] reported that the plant extract increases muscle basal glucose uptake and Al-Malki *et al.*[13] reported antioxidant activity. However, it could be due to the regeneration of β-cells from the remnant pancreatic cells available after partial damage due to STZ. From the percentage changes in available serum insulin between the treated and untreated diabetic rats groups, one could infer that the increased in available serum insulin of rats groups under the fractionated extract treatment of *Balanites aegyptiaca* fruit-mesocarps may be due to β-cells regeneration from the available pancreas at the end of day 28 of the treatment. Studies have shown that *Balanites aegyptiaca* is rich in different biologically active phytochemicals[11,41,42].

Insulin is a potent inhibitor of lipolysis. During diabetes, the activity of lipase enzyme increases lipolysis and release more free fatty acids in the circulation because of lack of insulin[43]. Increase in fatty acid concentration in turn increases the β-oxidation of fatty acids by increasing the activity of HMG-CoA reductase for production of more cholesterol[44,45]. Insulin also increases the receptor-mediated removal of LDL-cholesterol and decrease in activity of insulin during diabetes causes hypercholesterolemia[43]. The level of reduction of serum cholesterol levels was significantly higher in the aqueous fraction treated group when compared to the group treated with metformin or ethyl acetate fractions. This observation signifies that aqueous fraction contained insulin stimulating components that inhibit lipolysis. However, in another perspective, one could suggest the inhibition of HMG-CoA reductase activity by the plant, since the study has shown an increased HMG-CoA reductase activity in diabetic

rats[45,46]. The percentage of available serum insulin observed in the experimentally induced diabetic rats group in this study agrees with the previous model of Type 1 diabetes[20,47,48].

Hyper-triglyceridemia associated with diabetic condition is a consequence of uninhibited actions of pancreatic lipase[49]. In this study, the administration of aqueous and ethyl acetate fractions of ethanolic extract of BAFM 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 and, in turn, leads to the observed decreased in plasma triglycerides level[48-51].

Albumin, globulin, hemoglobin, collagen, crystalline proteins may undergo non-enzymatic glycation when they are exposed to excess glucose in hyperglycemic state[52,53]. The observed decrease in albumin among the diabetic control group may be due to albuminuria which are important clinical markers of diabetic nephropathy or glycation and might also be due to increased protein catabolism[54-56]. Treatment of diabetic rats with the extract-fractions of BAFM improved the serum albumin levels. Glycated haemoglobin ($HbA1_c$) test may not depict the exact picture of blood glucose fluctuation in a short duration studies when compared to fructosamine test that could reveal the status of glycaemic control over a period of 2-3 weeks due to their shorter life span (approximately 17-20 days)[57]. For this reason, fructosamine was measured to access the glycemic changes in this study considering the study duration. Low level of fructosamine in diabetic treated rats may be ascribed to the restoration of blood glucose levels during the treatment or inhibition of glycation by *Balanites aegyptiaca* extract. Singh *et al.*[58] have reported the inhibition of glycation by eugenol, a phenolic compound from plant.

Elevation of serum aminotransferases (ALT and AST) activities in streptozotocin-induced diabetic rats in this study indicated an excessive release of such enzymes from the liver cells into the blood circulation. It was reported that there is an inverse relationship between the liver activity and the level of enzymes in serum which may reflect damage to the hepatic cells due to hepatotoxic effect of streptozotocin[59,60]. Diabetic rats treated with fractions from ethanol extract of *Balanites aegyptiaca* fruit-mesocarp recorded a decrease in the AST enzyme activity, suggesting inhibitory effect of the extract-fractions. However, elevated activity of ALT may suggest the failure to prevent leakage of the liver enzymes by *Balanites aegyptiaca* fruit-mesocarp ethanol extract-fractions due to their inability in membrane stabilizing activity since plant extract had prevented leakage of intracellular enzymes via membrane stabilizing effects[49].

Type 1 diabetes mellitus is characterized by severe loss of body weight that may result from relative or absolute deficiency of insulin due to defective β-cells[61]. The loss of weight in STZ-induced diabetic rats might be accompanied by increased breakdown of muscle proteins (for the provision of gluconeogenic amino acids) as suggested by Bastaki[61], which indicates that the degradation of structural protein is a contributing factor towards weight loss. Continuous treatments of STZ-induced diabetic rats with the fractions of ethanolic extract of *Balanites aegyptiaca* fruit-mesocarp significantly prevented body weight loss.

Decrease in PCV levels of diabetic rats are the result of RBC's haemolysis[62]. Increased lipid peroxidation in the RBC's is due to an inhibition or changes in the activity of non-enzymatic and/or enzymatic components of the oxidative system (reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities[63]. Improved PCV level in this study indicates that ethanol extract-fractions of *Balanites aegyptiaca* fruit-mesocarp may contain compound(s) that have the ability to prevent lipid peroxidation or mop up free radicals. Nadro and Samson[62] have reported significant protective effect of *Balanites aegyptiaca* kernel cake on PCV levels in alloxan-induced diabetic rats.

A major limitation of this study was the unavailability and accessibility to high techniques such as high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS) that would have assisted in the further fractionation of the sub-fractions for possible identification and structural elucidation of the bioactive molecules.

CONCLUSION

Both aqueous and ethyl acetate fractions of ethanolic extract of BAFM exert antihyperglycemic and antilipidemic effects. However, the aqueous fraction was more potent, suggesting that the bioactive component(s) present in the fruit-mesocarp of *Balanites aegyptiaca* are polar in nature. This study has shown that extract-fractions of *Balanites aegyptiaca* fruit exhibit antidiabetic activity on type 1 diabetes and this calls for further screening on type 2 diabetes.

SIGNIFICANCE STATEMENT

This study has identified the nature of the anti-diabetic bioactive components present in *Balanites aegyptiaca* fruit-mesocarp as polar in nature, which in no doubt, many other researchers were not able to explore. Hence, it will be of

help to further researches that would uncover the unknown bioactive components present in *Balanites aegyptiaca* fruit-mesocarp, an outcome that may include development of a new class of drug (and possibly other combinations) for the management of diabetes mellitus.

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