

Hypoglycemic Effects of *Balanites aegyptiaca* Fruits' Ethanolic Extract and its Fractions in Streptozotocin-Induced Diabetic Rats

*Oluwayemisi Beatrice Ibitoye, Faiza Isa Atahiru

Antioxidants, Redox Biology and Toxicology Research Group,
Department of Biological Sciences,
Faculty of Natural & Applied Sciences,
Al - Hikmah University,
P. M. B 1601, Adewole,
Ilorin,
Nigeria.

Email: oluwayemi.ojewuyi@alhikmah.edu.ng

Abstract

Diabetes has continuously generated global havoc in the last few years, therefore there is need to explore different measures such as the use of medicinal plants to manage this disease. In this study, *Balanites aegyptiaca* fruits, which has been used by traditionalist over a period of time was investigated in Streptozotocin-induced rats. *Balanites aegyptiaca* fruits were processed and extraction was done using ethanol, the extract was partitioned using N-hexane and N-butanol. Thirty six male rats were randomized into six groups (group A-F) having six rats each, group A served as the control (normal rats), groups B-F were induced with 45 mg/kg bodyweight Streptozotocin, group B was given distilled water while Groups C-F were treated using metformin, the ethanolic extract, n-butanol and n-hexane fractions respectively for 14 days. During the experiment, fasting blood glucose level was taken, an oral glucose tolerance test was performed on the 15th day, after which the rats were anesthetized with diethyl ether fume and sacrificed, the blood collected was used for glycated hemoglobin assay and the pancreas were excised for histopathological assessment. Treatment with the ethanolic extract and partitioned fractions significantly reversed the hyperglycemia mediated changes. The oral glucose tolerance test demonstrated improved glucose utilization, the HbA1c level were normalized and histopathological assessment showed reversal of pancreatic cell degeneration in treated groups when compared to the control ($p < 0.05$). Hence, the study proves that the ethanolic extract and fractions of *B. aegyptiaca* fruit can adequately manage Diabetes mellitus and this supports the traditional claim of its use.

Keywords: *Balanites aegyptiaca*, Diabetes, Hyperglycemia, Hypoglycemia, Streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is a silent epidemic affecting millions of humans round the globe. It is a complex metabolic disorder with underlying causes such as chronic hyperglycemia accompanied by disturbance in carbohydrate, fat and protein metabolism (Dilworth *et al.*, 2021). Based on etiology, diabetes is caused when the body cease to use glucose effectively due to low insulin (Type1 DM) or glucose insensitivity (Type 2 DM) (Ibitoye *et al.*, 2021), apparently leading to high glucose levels (Artur *et al.*,

*Author for Correspondence

2021: Aiesha *et al.*, 2023). Diabetes mellitus is among the most common and resource-intensive chronic diseases in the world (Xiang *et al.*, 2021). The International Diabetes Federation (IDF) reported that the prevalence of DM increased from 422 million in 2014 to 537 million in 2021. This rise is expected to reach 643 million by 2030 and 783 million by 2045 (IDF, 2021). Although, the figure is a gross underestimation, as there is a larger number of people living with diabetes unknown to the World Health Organization (Solomon *et al.*, 2023). Poorly managed cases of diabetes can lead to symptoms such as increased thirst and hunger, frequent urination, fatigue, blurred vision and ulcerations (Dwibedi and Pandey, 2020), which can cause severe consequences as heart diseases, stroke, neuropathy, retinopathy, amputations and kidney damage or failure (American Diabetes Association ADA, 2023).

Healthy diet, life style modification, regular exercise and weight management can help in the management of DM. Medicinal plants have proven to be effective in the management of blood glucose levels and improved glucose metabolism (Roy *et al.*, 2022; Yedjou *et al.*, 2023). It is therefore obvious that in the 21st century medicinal plants and their bioactive agents/derivatives offer opportunities for disease management, especially where current orthodox treatment methods have often failed (Picot *et al.*, 2019). *Balanites aegyptiaca* (Desert date) is one amongst such plants used locally for the treatment of DM. It is rich in flavonoids, polyphenols, alkaloids and saponin which proffer potent antioxidant effects (Isa *et al.*, 2023). It is majorly found in the Sahel part of Nigeria and it is called Aduwa in Hausa, Utaz in Igbo and Teji in Yoruba (Tula *et al.*, 2014; Wakawa *et al.*, 2018; Isa *et al.*, 2023). *B. aegyptiaca* fruit and seed aqueous extracts exhibit potential anti-hyperglycemic and anti-hyperlipidemic effects (Abou Khalil *et al.*, 2016; Zaky *et al.*, 2022). Several studies have reported the antidiabetic potential of the seed extract however, there is limited scientific evidence on the antidiabetic properties of the solvent-fractions of *B. aegyptiaca* fruit as a probe to its mechanism of action in the management and treatment of DM. This study therefore aim to evaluate the antidiabetic potential of the solvent-fractions of the ethanolic extract of *B. aegyptiaca* fruit.

MATERIALS AND METHODS

Reagents and Kits

All reagents used were of analytical grade.

Collection of sample

The *Balanites aegyptiaca* fruits were bought from Kawo Market, Kaduna State, Nigeria. The fruits were then identified and authenticated at the University of Ilorin Herbarium, where a voucher number UILH/001/1801/2024 was given to the plant.

Preparation of *Balanites aegyptiaca* ethanolic extracts

The *Balanites aegyptiaca* fruits were peeled, the seeds were removed and rinsed with clean water to remove residues. After which the fruits were air-dried at room temperature until a constant weight was achieved. The dried fruits were pulverized into a coarse powder using an electric blender.

The ethanolic extract was prepared using the method described by Abul Kalam & Wan-Mohd (2020). About 800g of the sample was extracted by cold maceration with ethanol (5:1, solvent: solid ratio) for 72 hours at room temperature, then filtered into a sterile round bottom flask using adsorbent cotton wool and filter paper (Whatman No. 1). The extraction

procedure was repeated three times to ensure the highest percentage yield of ethanol-soluble compounds from the fruit powder. The ethanol extract was concentrated using a rotary evaporator.

Partitioning of *Balanites aegyptiaca* ethanolic Extract

Ethanolic extract of *B. aegyptiaca* was partitioned using column chromatography. N-hexane and N-butanol was used to partition the ethanolic extract as described by Ibitoye *et al.* (2018).

Laboratory Animals

Thirty-six male wistar rats (148.9 ± 30 g) were obtained from Research Fulcrum Laboratory, Tanke, Ilorin, Kwara State. All the animals were housed in cages and maintained in a standardized animal house under room temperature. Principles of laboratory animal care guidelines were followed in handling the animals (National Institutes of Health, 2023).

Induction of diabetes

A single-dose (45 mg/kg body weight) of intraperitoneal injection of freshly prepared (in citrate buffer 0.1 M pH 4.5) Streptozotocin was used to induce diabetes in overnight fasted rats. The blood glucose was taken after 72 hours, rats with blood glucose above 238 mg/dL were considered diabetic (Ghasemi and Jeddi, 2023).

Animal Grouping

Thirty-six male rats (148.9 ± 30 g), after two weeks of acclimatization, were randomly grouped into six groups of 6 rats each, and treated as follows (Solomon *et al.*, 2023):

Group A received distilled water only; Group B received streptozotocin and distilled water; Group C received streptozotocin and treated with metformin (reference drug); Group D received streptozotocin and treated with 250 mg/kg body weight of the ethanolic extract; Group E received streptozotocin and treated with 150 mg/kg body weight of partition portion of N-butanol; Group F received streptozotocin and treated with 100 mg/kg body weight of the partition portion of N-hexane.

Fasting Blood Glucose

Fasting blood glucose (FBG) levels were taken at intervals (0, 3rd, 6th, 9th, 12th and 15th day) to monitor sustained hyperglycemia and the effect of the treatment during the experiment, using a blood glucometer. Blood was drawn from the tail vein using a sterilized needle, and rats with FBG levels >250 mg/dL were considered diabetic (Bhanu *et al.*, 2021).

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed at the end of the 15th day, following the procedure used by Bhanu *et al.* (2021). Rats in all the experimental groups were orally administered glucose (2 mg/kg of BW) after overnight fasting. Glucose was given orally to the rats. Blood glucose levels were measured at 0, 30, 60 and 120 minutes using blood from the tail vein.

Sample Collection and Preparation

Rats were sacrificed after anesthetization with diethyl ether and blood samples were individually collected via cardiac puncture into plain bottles and allowed to clot, centrifuged, and the resultant serum was harvested for biochemical assay. The liver, kidney

and pancreas were excised via abdominal incision and processed for further studies (Solomon *et al.*, 2023).

Glycated Hemoglobin

Glycated hemoglobin (HbA1c) was measured using cation exchange high-performance liquid chromatography (HPLC) as described by Mohammed *et al.* (2021). Briefly, the HbA1c was separated from other hemoglobin fractions using a cation exchange column, and the absorbance was measured at 415 nm. The HbA1c results were expressed as a percentage of total hemoglobin, and the reference range was established using healthy Wistar rats.

Histopathology

Liver and pancreatic tissues isolated from the experimental rats were washed and fixed immediately in 10% formalin solution. Histopathological studies were conducted by dehydration of formalin-fixed tissues in a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. The paraffin blocks were sectioned into 5- μ m thickness using a microtome. The sections were fixed on a gelatin-coated slides and finally stained with hematoxylin and eosin. The pathological changes in the tissues were observed under a light microscope according to a previously described method (Ismail *et al.*, 2018).

Data analysis

The data generated from the study was presented as mean $\pm$ standard error of the mean of three replicates and subjected to one-way Analysis of Variance (ANOVA). The data was considered statistically different at ($p < 0.05$) using GraphPad Prism version 8.0.2 (GraphPad Software, Inc., San Diego, California, United States).

RESULTS

Fasting Blood Glucose

There was no significant difference ($p < 0.05$) in the fasting blood glucose of all groups at day 0 (Table 1). The fasting blood glucose of rats in the untreated group was significantly higher ($p < 0.05$) compared to the control throughout the period of the study. The resulting elevated fasting blood glucose was significantly ameliorated in the extract-treated groups. Moreover, the glucose-lowering effect was significantly higher in the n-hexane extract-treated group compared to the n-butanol extract and the ethanolic extract groups.

Table 1: The Blood glucose level of the Streptozotocin-induced diabetic rats treated with *Balanites aegyptiaca* fruits ethanolic extract and its fractions

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Blood glucose (mg/dL)						
A - Control	113.25 ± 7.04 ^a	107.50 ± 5.91 ^a	100.25 ± 6.85 ^a	98.55 ± 6.85 ^a	100.00 ± 5.58 ^a	97.23 ± 5.34 ^a
B - Diabetic rats + Distilled water	109.00 ± 5.01 ^b	438.00 ± 8.29 ^b	439.33 ± 8.41 ^b	435.65 ± 3.35 ^b	430.60 ± 6.28 ^b	433.25 ± 8.49 ^b
C - Diabetic rats + 50 mg/kg b.w metformin	103.50 ± 5.95 ^b	376.67 ± 45.99 ^c	219.83 ± 5.93 ^c	168.80 ± 5.57 ^c	142.75 ± 5.17 ^c	100.75 ± 6.32 ^a
D - Diabetic rats + 250 mg/kg b.w ethanolic extract	102.00 ± 3.68 ^b	459.17 ± 29.64 ^b	237.33 ± 17.87 ^c	163.60 ± 6.07 ^c	123.75 ± 3.45 ^d	99.75 ± 2.21 ^a
E - Diabetic rats + 150 mg/kg b.w N-butanol fraction	114.50 ± 3.14 ^a	398.83 ± 4.71 ^c	209.33 ± 9.44 ^c	141.40 ± 2.16 ^d	110.50 ± 5.98 ^a	87.50 ± 1.85 ^a
F - Diabetic rats + 150 mg/kg b.w N-hexane fraction	117.50 ± 6.11 ^a	344.33 ± 2.99 ^d	207.33 ± 2.85 ^c	124.60 ± 3.68 ^e	115.50 ± 1.89 ^a	100.75 ± 3.47 ^a

KEY: b, w - body weight

Values are expressed as mean of 6 determinations ± standard error of mean (SEM) and are significantly different at $p < 0.05$.

Oral Glucose Tolerance

The glucose tolerance of the untreated group was significantly lower ($p < 0.05$) compared to the normal control group. This trend was significantly alleviated in all the extract-treated groups (Table 2). Glucose tolerance was significantly higher in the n-hexane extract group than others.

Table 2: Glucose tolerance of Streptozotocin-induced diabetic rats treated with *Balanites aegyptiaca* fruits ethanolic extract and its fractions

Group	0 min	30 mins	60 mins	120 mins
Blood glucose (mg/dL)				
A - Control	97.23 ± 5.34 ^a	93.00 ± 6.83 ^a	86.50 ± 7.10 ^a	76.75 ± 7.95 ^a
B - Diabetic rats + Distilled water	433.25 ± 8.49 ^b	454.75 ± 28.47 ^b	439.50 ± 27.62 ^b	426.25 ± 27.47 ^b
C - Diabetic rats + 50 mg/kg b.w metformin	100.75 ± 6.32 ^a	104.75 ± 6.02 ^a	88.50 ± 7.24 ^a	70.25 ± 7.15 ^c
D - Diabetic rats + 250 mg/kg b.w ethanolic extract	99.75 ± 2.21 ^a	108.75 ± 2.39 ^a	86.25 ± 2.49 ^a	69.50 ± 2.90 ^c
E - Diabetic rats + 150 mg/kg b.w N-butanol fraction	87.50 ± 1.85 ^a	104.75 ± 2.39 ^a	77.00 ± 2.42 ^c	61.50 ± 1.04 ^c
F - Diabetic rats + 150 mg/kg b.w N-hexane fraction	100.75 ± 3.47 ^a	108.50 ± 3.89 ^a	83.50 ± 3.58 ^a	65.25 ± 2.87 ^c

KEY: b. w - body weight

Values are expressed as mean of 6 determinations ± standard error of mean (SEM) and are significantly different at $p < 0.05$.

Glycated Hemoglobin

The percentage of glycated hemoglobin in the untreated group was significantly higher ($p < 0.05$) compared to the normal control group. This diabetes-mediated change decreased in all the extract-treated groups (Figure 1).

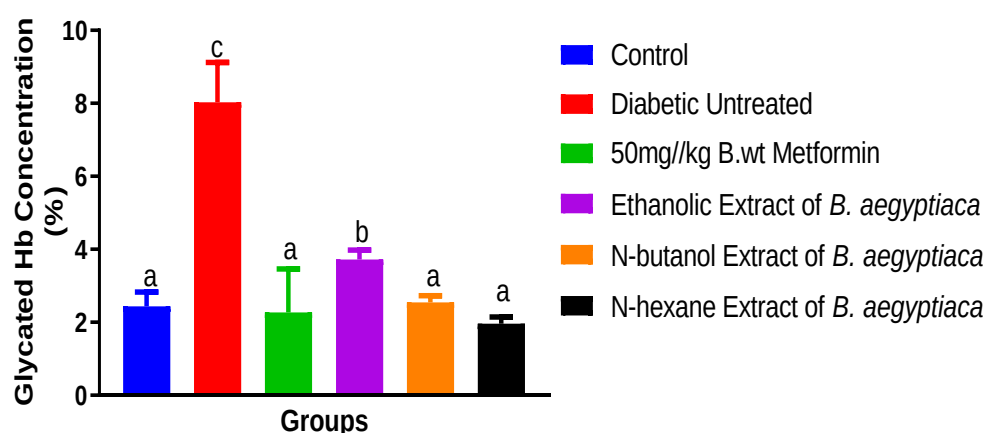


Figure 1: Glycated Haemoglobin of Streptozotocin-induced diabetic rats treated with *Balanites aegyptiaca* fruits ethanolic extract and its fractions

Values are expressed as mean of 6 determinations $\pm$ standard error of mean (SEM) and are significantly different at $p < 0.05$.

Histopathology of the Pancreas

The Plates (A - F) show the histopathological assessment of the rats' pancreas. There were no features of degeneration in the pancreatic tissues of the normal control pancreas (Plate A). The features in the untreated group were suggestive of degenerative islet of Langerhan (red arrow) in lymphoid bed (black arrow), none pancreatic feature seen (Plate B). The tissues in the pancreas of the metformin group had normal architecture (Plate C). The tissues in the ethanolic-extract, n-butanol and n-hexane groups show exocrine glands (black arrow) and Langerhans cells which appeared in moderated and active pattern (Plate D-F).

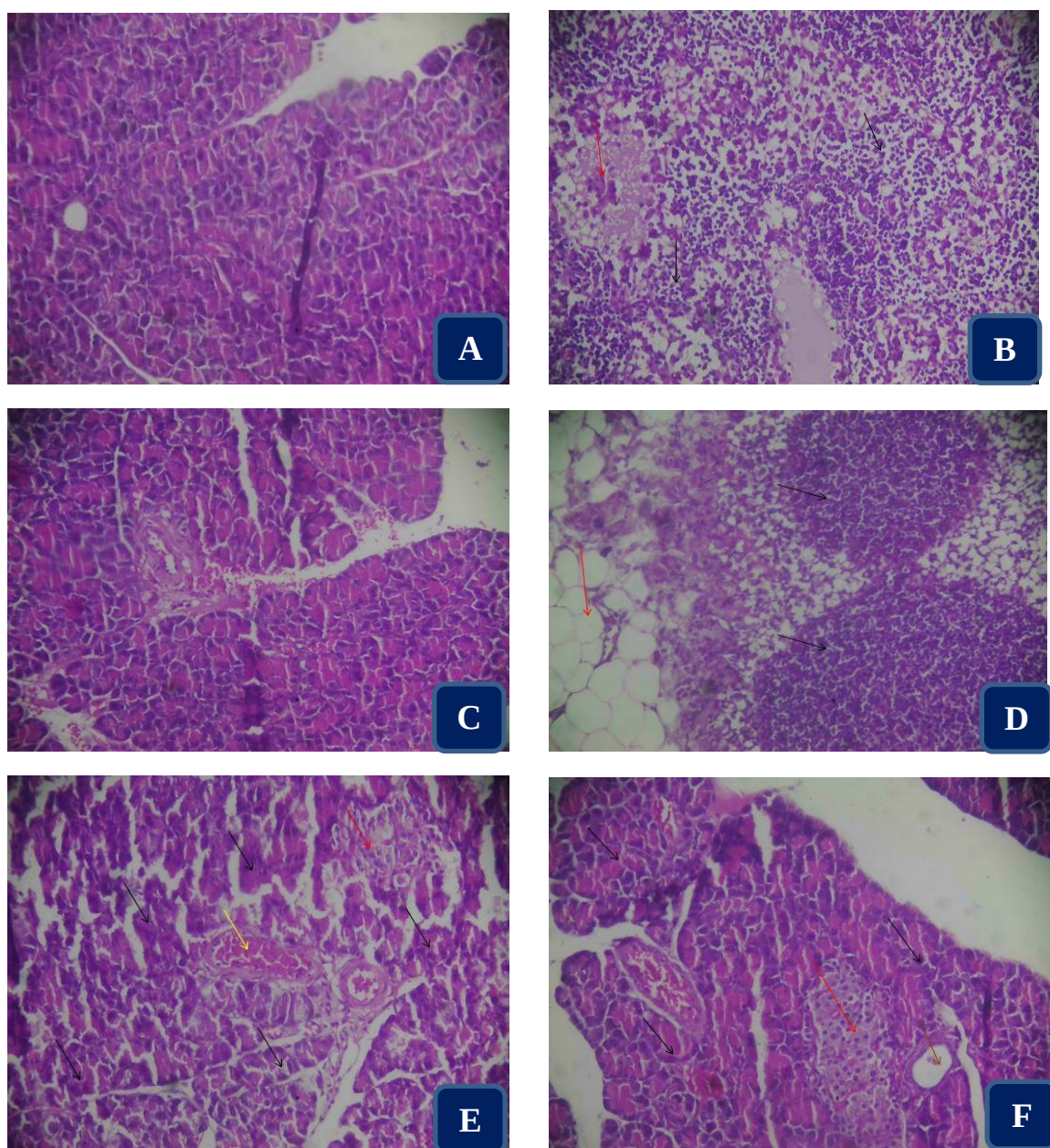


Plate (A to F). Histopathology of the pancreas of Streptozotocin-induced diabetic rats treated with *Balanites aegyptiaca* fruits ethanolic extract and its fractions

DISCUSSION

Research has validated the ability of medicinal plants to lower blood glucose levels and improve insulin sensitivity, an approach that is widely used for managing diabetes (Kumar *et al.*, 2011). Due to the perceived safety, natural origin, less side effects, as well as cost-effectiveness and increased accessibility, medicinal plants are favored for the illness management in most parts of the world (Gao *et al.*, 2018). Medicinal plants promote a holistic approach to health by addressing the underlying causes of diabetes (Kumar *et al.*, 2014).

Balanites aegyptiaca, also known as the desert date, is an underutilized fruit-yielding tree native to Africa (Murthy *et al.*, 2021), whose fruit, leaves, and seeds have been used in traditional medicine for various purposes, including antidiabetic activity, as studies have shown that extracts from *B. aegyptiaca* possess antidiabetic properties, improving insulin sensitivity and reducing blood glucose levels (Al-Malki *et al.*, 2015). Additionally, the plant contains antioxidants, which help protect against oxidative stress and inflammation (Al-Malki *et al.*, 2015), and has been traditionally used to treat inflammatory conditions, with its extracts exhibiting anti-inflammatory properties (Gao *et al.*, 2018). Furthermore, *B. aegyptiaca* has been found to exhibit antimicrobial activity against various microorganisms (Anani *et al.*, 2015). Overall, *B. aegyptiaca* may be a potential medicinal plant for managing diabetes mellitus, given its antidiabetic and antioxidant properties.

This study provides important insights into the anti-diabetic potential of the ethanolic extract of *Balanites aegyptiaca* and its partitioned fractions compared to a standard treatment. Polar solvents like ethanol and n-butanol are known to extract a broader range of phytochemicals, including polyphenols and flavonoids, which have documented anti-diabetic properties (Tiwari and Rao, 2002; Hossen *et al.*, 2020).

Fasting blood glucose levels (FBG) is a critical indicator of glucose metabolism and diabetes management, and reducing FBG levels can help prevent long-term complications of diabetes (ADA, 2020). The fruit's ethanolic extract and its fractions were investigated for their effects on fasting blood glucose (FBG) levels. The results showed that the ethanolic extract was the most successful in lowering FBG levels, followed by the n-butanol fraction, which was comparable with standard treatment, and the less successful n-hexane fraction (ADA). From day 3 to day 12, the FBG levels of the treated and untreated groups were considerably lower, respectively, indicating the potential of the extracts as natural anti-diabetic therapy. The polarity of the solvent was shown to be the cause of the extracts' differing efficacies; ethanol was found to extract a wider variety of phytochemicals with anti-diabetic qualities, such as polyphenols, flavonoids, and saponins (Kumar *et al.*, 2014). The superior efficacy of the ethanolic extract suggests a synergistic effect of multiple compounds, possibly acting on different biochemical pathways to lower blood glucose more effectively.

The Oral Glucose Tolerance Test (OGTT) is a diagnostic tool used to assess how well an organism can manage glucose after an oral glucose load, providing insights into insulin sensitivity and glucose metabolism (WHO, 2006). The results presented in Table 3 reveal that the rats in the treated groups were able to manage glucose properly within few hours when compared to the control group while the management of glucose in the untreated group was very poor, this might be as a result of the ability of the fruit extracts to regenerate pancreatic β -cells and secrete insulin, or a result of the extracts' ability to stimulate the release of bound insulin from β -cells by inhibiting ATP-sensitive K^+ channels which comes in line with the findings of Alam *et al.*, (2023).

The assessment of glycated hemoglobin (HbA1c) is a crucial marker for assessing the efficacy of the treatment of long-term glucose control and it is formed when glucose attaches to hemoglobin in the blood, and its levels reflect average blood glucose concentrations over time (Alam *et al.*, 2023). The findings from this study are supported by prior research showing that certain plant extracts can lower HbA1c levels, though some extracts may be more effective in acute glucose management than in long-term control (Al-Attar and Shawush, 2014). The results from this study show that the fruit has a possible hypoglycemic effect.

Histopathological analysis of pancreatic tissues give insights into the structural integrity and functional state of the pancreas under different experimental conditions (Yang *et al.*, 2020). Streptozotocin causes degeneration in Langerhans islet beta cells in the pancreas and induces experimental diabetes in rats within three days (Akbarzadeh *et al.*, 2007). The pancreatic tissues from the normal control group displayed no signs of degeneration. The islets of Langerhans, responsible for insulin production, were intact and well-organized, indicating healthy pancreatic architecture and function. The absence of degenerative features suggested that the normal control pancreas is functioning optimally and serves as a baseline for assessing pancreatic damage or improvements in other treatment groups. Contrarily, the diabetic untreated group exhibited features indicative of degenerative changes in the islets of Langerhans and a notable lymphoid infiltration. The degeneration of islets suggested damage or dysfunction, likely related to the chronic effects of untreated diabetes. Treatment with the extracts in the other groups was able to effectively mitigate the degenerative changes and supports pancreatic health, maintaining its structural and functional integrity.

CONCLUSION

The ethanolic extract and fractions of *Balanites aegyptiaca* improved glycemic control, mitigated diabetic complications and preserved the pancreatic architecture and function. The study therefore, suggests that *Balanites aegyptiaca* extracts possess antidiabetic properties and may be a useful adjunct or alternative therapy for managing diabetes and its complications.

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