



Research Article

Antihyperglycemic Effect of *Balanite aegyptiaca* Aqueous Fruit Pulp Extract on Streptozotosin Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Despite the availability of synthetic antidiabetic drugs, their high cost and adverse effects have encouraged the exploration of safer, plant-based alternatives. *Balanites aegyptiaca* (desert date) is traditionally used to treat diabetes, but its scientific validation remains limited. This study evaluated the antihyperglycemic effect of the aqueous fruit pulp extract of *Balanites aegyptiaca* in streptozotocin (STZ) induced diabetic rats. Diabetes was induced by intraperitoneal injection of nicotinamide (60 mg/kg) followed by STZ (60 mg/kg). Diabetic rats were treated orally for two weeks with *B. aegyptiaca* extract (100, 200, and 400 mg/kg) and glibenclamide (20 mg/kg). Fasting blood glucose, lipid profile, liver function, haematological parameters, and pancreatic histology were analysed using standard procedures. The extract significantly reduced fasting blood glucose and improved lipid profile by lowering total cholesterol, triglycerides, and LDL while increasing HDL. Liver enzyme activities (AST, ALT, and bilirubin) were normalized, haematological indices improved, and histopathological examination showed partial regeneration of pancreatic β -cells. *B. aegyptiaca* aqueous fruit pulp extract exhibits antihyperglycemic, hypolipidemic, hepatoprotective, and haematoprotective effects in diabetic rats, supporting its traditional use and highlighting its potential as a safe, affordable phytomedicine for diabetes management.

Keywords: Antihyperglycemic; Aqueous extract; *Balanites aegyptiaca*; Diabetes; Streptozotosin

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both (Andrew *et al.*, 2018). It disrupts the normal metabolism of carbohydrates, fats, and proteins, and if left uncontrolled, can result in progressive damage to multiple organ systems particularly the blood vessels, kidneys, eyes, and nerves. According to the World Health Organization (2024), diabetes arises either from insufficient insulin production by the pancreas or the body's inability to use insulin

effectively. Diabetes mellitus is broadly categorized into two main types: Type 1 and Type 2 diabetes. Type 1 diabetes (T1DM) is characterized by autoimmune destruction of pancreatic β -cells, leading to an absolute insulin deficiency. It often manifests in childhood or adolescence and requires lifelong insulin therapy. On the other hand, Type 2 diabetes mellitus (T2DM) which accounts for over 90% of global diabetes cases is primarily associated with insulin resistance and progressive β -cell dysfunction (ADA, 2022). The global prevalence of diabetes has increased dramatically over recent

decades, driven by urbanization, sedentary lifestyles, unhealthy diets, obesity, and aging populations. The International Diabetes Federation (IDF) estimates that approximately 425 million people are currently living with diabetes worldwide, with nearly half remaining undiagnosed (Andrew *et al.*, 2018). In 1990, the global prevalence among adults aged 18 and older was 7%; by 2022, this figure had doubled to 14%. In 2021, diabetes directly caused approximately 1.6 million deaths, with 47% occurring in people under 70 years of age. Additionally, high blood glucose contributed to around 11% of cardiovascular deaths and 530,000 deaths from kidney disease (WHO, 2022).

Although the current synthetic antidiabetic medications have many advantages, they also have a lot of negative side effects, so there is a need for alternative antidiabetic agents with fewer or no harmful side effects. In recent years, new active medicines have been extracted from plants that have more effective antidiabetic activity than oral chemical hypoglycemic drugs used in proven therapy (Zaky *et al.*, 2022). Medicinal plants contain a variety of bioactive compounds that have multiple activities in insulin production, insulin action, or both. For the treatment of diabetes mellitus, Eskander and WonJun described a variety of Egyptian plant and herb prescriptions that fall into different families. African nations have long utilized *Balanites aegyptiaca* (L.) Delile, a member of the Zygophyllaceae family, as an antihelmintic and to treat jaundice. Herbalists in the Egyptian market offer the fruit as an antidiabetic, and it is used as an oral anti-hyperglycemic in Egyptian traditional medicine (Zaky *et al.*, 2022).

Given the increasing burden of diabetes, especially in resource-limited settings, and the limitations of existing pharmacotherapies, there is a compelling rationale to explore indigenous medicinal plants like *Balanites aegyptiaca* for their therapeutic potential and scientific validation. This study, therefore, aims to evaluate the antidiabetic effect of aqueous fruit pulp extract of *Balanites aegyptiaca* in streptozotocin induced diabetic rats, with the objective of identifying safe, effective, and affordable alternatives for managing type 2 diabetes mellitus.

MATERIALS AND METHODS

Materials

Streptozotocin, nicotinamide and Glibenclamide, normal saline, distilled water, sodium chloride, phosphate-buffered saline (PBS, pH 7.5), and 10% formalin, 2,4-dinitrophenylhydrazine (DNPH), Sodium hydroxide, chloroform and bromocresol green were purchased from a standard commercial supplier.

Experimental Animals

The animals were purchased from animal house of Kebbi state University of science and Technology Aliero and were housed in the metallic cages with normal provision of food, water and ambient circumstances, normal 12-hours light and dark period were also maintained, the animals were allowed to acclimatize for two weeks. The university's relevant ethics committee granted approval for the work. The guidelines conform to the National Institutes of Health's guidelines for the care and use of laboratory animals (Edition, 2011)

Induction of Diabetes

After fasting for 16 hours, Diabetes mellitus was experimentally induced in albino rats via an intraperitoneal (IP) injection of nicotinamide (NA) 60 mg/kg body weight (b.w.) before the IP injection of streptozotocin (STZ) 60mg/kg (b.w.) to 16-hour fasted rats (Zaky *et al.*, 2022). The rats were tested for serum glucose levels 48 hours after STZ was injected. The overnight-fasted (10–12 h) animals were given glucose (3 g/kg b.w) via an oral gavage. The blood samples were taken from lateral tail vein after 2 h of oral administration and measured using glucometer (ACCU-CHEK® Active, Roche Diagnostics, and Mannheim, Germany). Rats with glucose levels >11 mmol/L were recognized as having diabetes and were used for the study.

Preparation of Extract

Balanite aegyptiaca fruit was purchased from central market Birnin Kebbi, the fruits were soaked in water for 48 hours and then filtered. By using rotary evaporator, the extract was concentrated by removing excess water.

Research Design

The rats were allocated into 6 groups and were given extract and standard drug daily for two weeks via oral gavage.

Group I (Normal control): This group was assigned as the normal control group, and rats included in this

group were given distilled water daily (5 mL/kg b.w./day).

Group II (Diabetic Untreated); This group was assigned as the diabetic control group, and the diabetic rats within this group were given distilled water daily (5 mL/kg b.w./day).

Group III (glibenclamide-treated group); this group consisted of diabetic rats that were treated with glibenclamide 20mg/kg b.w.

Group IV (100mg/kg b.w. of extract); This group consisted of diabetic rats that were treated daily with *B. aegyptiaca* fruit extract at a dose level of 100 mg/kg b.w.

Group V (200mg/kg b.w of extract); This group consisted of diabetic rats that were treated daily with *B. aegyptiaca* fruit extract at a dose level of 200 mg/kg b.w.

Group VI (400mg/kg b.w. of extract); This group consisted of diabetic rats that were treated daily with *B. aegyptiaca* fruit extract at a dose level of 400 mg/kg b.w.

Blood Sample Collection Procedures

Blood samples were collected using Hugo and Russel's method (Hugo and Russel, 2001). Rats were anesthetized with chloroform in a glass chamber, avoiding lethality to ensure blood flow. Each rat was secured on a workbench with pins, and a surgical blade incised the chest dorsoventrally. Blood was drawn from the beating heart via heparinized capillary tubes into sample bottles. A portion was centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for biochemical and haematological assays. Pancreas was excised, preserved in formalin for histopathology.

Biochemical Analysis

The serum ALT and AST were determined using reagent kit purchased from Spectrum for Diagnostic Industry (Egypt) following Reitman and Frankel's (1957) method, serum billurubin was determined using reagent kit purchased from Spectrum for Diagnostic Industry (Egypt) following Jendrassik and Grof's (1938) method, serum albumin was determine using bromocresol green method following Labcare Diagnostics Method, similarly serum protein was determined using Gornall *et al.*, (1949) method.

The serum cholesterol was determined using reagent kit purchased from Spectrum for Diagnostic Industry (Egypt) following Allain *et al.*, 1974 method,

serum triglyceride was determined using kit purchased from Spectrum for Diagnostic Industry (Egypt) following Bucolo and David (1973) method, serum HDL was determined using reagent kit purchased from Spectrum for Diagnostic Industry (Egypt) following Burstein *et al.*, (1970) method. LDL is calculated using the formula; $LDL\ Cholesterol\ (mg/dL) = Total\ Cholesterol - \frac{Triglycerides}{5} - HDL\ Cholesterol$ (Friedewald *et al.*, 1972)

Haematological Analysis

This was carried out using hematology auto analyzer machine (YSTE320A) from Shenzhen dynamic Biotechnology Company limited, Blood sample was well mixed as aspirated through an auto analyzer, and this counts the number and types of different cell within the blood.

Histopathological Evaluation of the Pancreas

The pancreas from each rat was rapidly excised after dissection and then fixed in 10% neutral buffered formalin for 24 h. The organs were routinely processed and sectioned at a thickness of 4 to 5 μ m. The sections of the pancreas were stained with hematoxylin and eosin for microscopic extermination (Bancroft & Gamble, 2002)

Data Analysis

Data were analyzed using the GraphPad Prism version 10 (GraphPad Software). One-way analysis of variance (ANOVA) was employed to assess differences between group means, with a p-value < 0.05 indicating statistical significance. Results were expressed as mean \pm standard deviation (SD) to quantify variability (Field, 2013).

RESULTS

Effect of Balanite aegyptiaca Aqueous Fruit Pulp Extract on the Body Weight of the STZ Induced Rats

Table 1 shows the body weight changes in all experimental groups from baseline to week 2. Untreated diabetic rats experienced progressive weight loss as observed from baseline to week 2, while treatment with *Balanites aegyptiaca* extract and glibenclamide significantly improved body weight in a dose-dependent manner, suggesting amelioration of diabetes-associated weight loss.

Effect of Balanites aegyptiaca Aqueous Fruit Pulp Extract on Blood Glucose Level of Streptozototin Induced Diabetic Rats.

The changes in fasting blood glucose levels of normal control, untreated diabetic control, glibenclamide treated, and extract-treated rats (100, 200, and 400 mg/kg b.w.) over a two-week period are presented in Table 2. At baseline, diabetic groups exhibited significantly elevated blood glucose compared to

normal controls. Treatment with *Balanites aegyptiaca* extract produced a dose-dependent reduction in blood glucose, with the 400 mg/kg dose showing the greatest effect, approaching values observed in the glibenclamide group.

Table 1. Changes in the Body Weight of the Experimental Rats

Groups (mg/kg b.w.)	Weight (g)		
	Baseline	Week 1	Week 2
Group I	125.33±0.58 ^a	149.73±0.67 ^b	157.29±0.87 ^c
Group II	180.5±0.86 ^a	170.83±0.83 ^b	155.3±0.33 ^c
Group III	120.33±0.58 ^a	139.93±0.87 ^b	149.6±0.52 ^c
Group IV	122.33±0.58 ^a	129.37±0.58 ^b	135.66±0.70 ^c
Group V	121.4±0.53 ^a	130.57±0.51 ^b	137.76±0.44 ^c
Group VI	121.33±0.58 ^a	140.18±0.57 ^b	150.02±0.45 ^c

Values are expressed as mean±standard errors of mean (SEM). Values with different superscripts across the row are statistically different ($p<0.05$) when compared with baseline. Group I = Normal control; Group II= Diabetic untreated; Group III= Glibenclamide treated; Group IV= 100mg/kg b.w. Extract; Group V= 200mg/kg b.w. extract; Group VI= 400mg/kg b.w. extract.

Table 2. Changes in the Blood Glucose Level of the Experimental Rats.

Groups (mg/kg b.w.)	Blood Glucose Level (mmol/dl)		
	Baseline	Week 1	Week 2
Group I	5.97±0.32 ^a	5.50±0.26 ^a	5.30±0.20 ^a
Group II	11.10±0.34 ^a	15.00±0.17 ^b	17.43±0.06 ^c
Group III	10.93±0.06 ^a	5.97±0.12 ^b	5.00±0.17 ^c
Group IV	12.93±0.06 ^a	9.03±0.12 ^b	7.67±0.06 ^c
Group V	12.17±0.06 ^a	8.27±0.06 ^b	6.93±0.06 ^c
Group VI	11.17±0.23 ^a	6.07±0.15 ^b	4.53±0.06 ^c

Values are expressed as mean±standard errors of mean (SEM). Values with different superscripts across the row are statistically different ($p<0.05$) when compared with base baseline. Group I Normal control; Group II= Diabetic untreated; Group III= Glibenclamide treated; Group IV= 100mg/kg b.w. extract; Group V= 200mg/kg b.w. extract; Group VI= 400mg/kg b.w. extract.

Effect of *Balanite aegyptiaca* on Liver Function Parameters.

Table 3 Summarizes the effect of two weeks of treatment with *Balanites aegyptiaca* extract on serum bilirubin, albumin, AST, ALT, and total protein levels in STZ-induced diabetic rats. Untreated diabetic controls showed significantly elevated bilirubin, AST, and ALT, along with reduced albumin and total protein, indicating hepatic dysfunction. Extract treatment significantly improved these parameters, particularly at 400 mg/kg, suggesting hepatoprotective effects.

Effect of *Balanite aegyptiaca* Extract on Lipid Profile.

Table 4 displays the serum lipid profile of all experimental groups after two weeks of treatment. Diabetic control rats had significantly higher total cholesterol, triglycerides, and LDL, with lower HDL compared to normal controls. Administration of *Balanites aegyptiaca* extract led to a dose-dependent decrease in total cholesterol, triglycerides, and LDL, with a corresponding increase in HDL levels, demonstrating hypolipidemic potential.

Effect of *Balanite aegyptiaca* Extract on Haematological Parameters.

Table 5 presents the haematological parameters (WBC, RBC, Hb, HCT (Hematocrit), MCV) across the experimental groups. Diabetes induction significantly reduced RBC, Hb, and HCT, while slightly elevating WBC and lowering MCV in the untreated group. Treatment with *Balanites aegyptiaca* extract improved RBC count, haemoglobin levels, and HCT in a dose-dependent manner, indicating positive effects on haematological health in diabetic rats.

Effect of *Balanite aegyptiaca* on the Histopathology

Histopathology of normal control, glibenclamide and 400mg/kg showed normal architecture of pancreas serous packed acini (AC) while untreated control, 100mg/kg and 200mg/kg Photomicrograph of pancreas tissue (H&E stains at X100) Showing architecture packed serous acini (ac) and islet of Langerhans (blue arrows) - containing α -cells and β -cells, Blood vessel (green arrow). serous acinar degeneration, atrophy and vacuolation (black arrow), Inflammatory cell infiltration (yellow arrow).

Table 3. Effect of *Balanite aegyptiaca* on Liver Enzymes

GROUPS mg/kg b.w.	Liver Function Test				
	BL (mg/dl)	Albumin (g/l)	AST (u/l)	ALT (u/l)	TP (g/dl)
Group I	0.67±0.01 ^a	41.07±0.41 ^a	17.54±0.34 ^a	18.18±0.42 ^a	9.81 ± 0.35 ^a
Group II	2.34±0.04 ^b	16.10±0.02 ^b	88.66±0.34 ^b	76.63±0.34 ^b	4.31 ± 0.38 ^b
Group III	0.99±0.01 ^c	33.35±0.09 ^c	30.38±0.58 ^c	32.01±0.38 ^c	8.96 ± 0.41 ^c
Group IV	1.72±0.0 ^d	23.67±0.13 ^d	43.26±0.47 ^d	45.20±0.34 ^d	5.60 ± 0.13 ^d
Group V	1.22±0.03 ^e	31.54±0.05 ^e	34.53±0.34 ^e	33.37±0.67 ^e	6.43 ± 0.16 ^e
Group VI	0.83±0.03 ^f	35.87±0.18 ^f	24.25±0.34 ^f	19.40±0.34 ^f	7.92 ± 0.14 ^f

Values are expressed as mean±standard errors of mean (SEM). Value with different superscripts down the column are statistically different (p<0.05). AST Aspartate aminotransferase; ALT Alanine aminotransferase; BL= Bilirubin; TP= Total protein; Group I= Normal control; Group II= Diabetic untreated; Group III= Glibenclamide treated; Group IV= 100mg/kg b.w. extract; Group V= 200mg/kg b.w. extract; Group VI= 400mg/kg b.w. extract

Table 4. Effect of *Balanite aegyptiaca* Extract on Lipid Parameters

GROUPS (mg/kg b.w.)	Lipid Profile (mg/dl)			
	Cholesterol	Triglyceride	HDL	LDL
Group I	81.30±0.28 ^a	90.99±0.28 ^a	79.07±0.49 ^a	46.98±0.72 ^a
Group II	183.60±0.64 ^b	171.11±0.32 ^b	24.64±0.05 ^b	166.35±0.58 ^b
Group III	105.51±0.07 ^c	100.41±0.49 ^c	55.92±0.43 ^c	71.14±0.52 ^c
Group IV	151.10±0.99 ^d	131.79±0.21 ^d	35.78±0.88 ^d	86.22±0.35 ^d
Group V	122.46±0.55 ^e	125.37±0.85 ^e	41.10±0.77 ^e	52.57±0.10 ^e
Group VI	87.87±0.07 ^f	113.77±0.86 ^f	50.05±0.07 ^f	38.13±0.53 ^f

Values are expressed as mean± standard errors of mean (SEM). Value with different superscripts down the column are statistically different (p<0.05) when compared with group I, group II, and group III. HDL= high density cholesterol; LDL= low density cholesterol; Group I= Normal control; Group II= Diabetic untreated; Group III= Glibenclamide treated; Group IV= 100mg/kg b.w. extract; Group V= 200mg/kg b.w. extract; Group VI= 400mg/kg b.w. extract.

Table 5. Effect of *Balanite aegyptiaca* Extract on Haematological Parameters

Group/Treatment (mg/kg B.W.)	Haematological Parameters				
	WBC (10 ⁹ /L)	RBC (10 ¹² /l)	HGB (g/dl)	HCT (%)	MCV (fl)
Group I	5.27±0.05 ^a	4.82±0.08 ^a	10.67±0.153 ^a	35.30±0.64 ^a	73.13±0.44 ^a
Group II	6.50±0.15 ^b	3.80±0.01 ^b	8.40±0.31 ^b	26.70±0.97 ^b	69.70±0.85 ^b
Group III	4.94±0.06 ^c	5.92±0.08 ^c	11.50±0.30 ^c	37.00±0.50 ^c	62.50±0.15 ^c
Group IV	4.30±0.07 ^d	5.93±0.07 ^{d,c}	11.33±0.15 ^{d,c}	37.60±0.45 ^{d,c}	63.40±0.15 ^d

Group V	3.10±0.08 ^e	5.43±0.06 ^e	10.57±0.458 ^{e,a}	33.70±0.90 ^e	61.90±0.69 ^e
Group VI	4.94±0.06 ^{f,c}	6.46±0.02 ^f	12.30±0.208 ^f	40.20±0.76 ^f	62.10±0.60 ^{f,c}

Values are expressed as mean± standard errors of mean (SEM). Values with different superscripts down the column are statistically different ($p < 0.05$) when compared with group I, group II, and group III. WBC= White blood cell, RBC= Red blood cell, HGB= haemoglobin, HCT=, MCV= mean corpuscular volume; Group I= Normal control; Group II= Diabetic untreated; Group III= Glibenclamide treated; Group IV= 100mg/kg b.w. extract; Group V= 200mg/kg b.w. extract; Group VI= 400mg/kg b.w. extract.

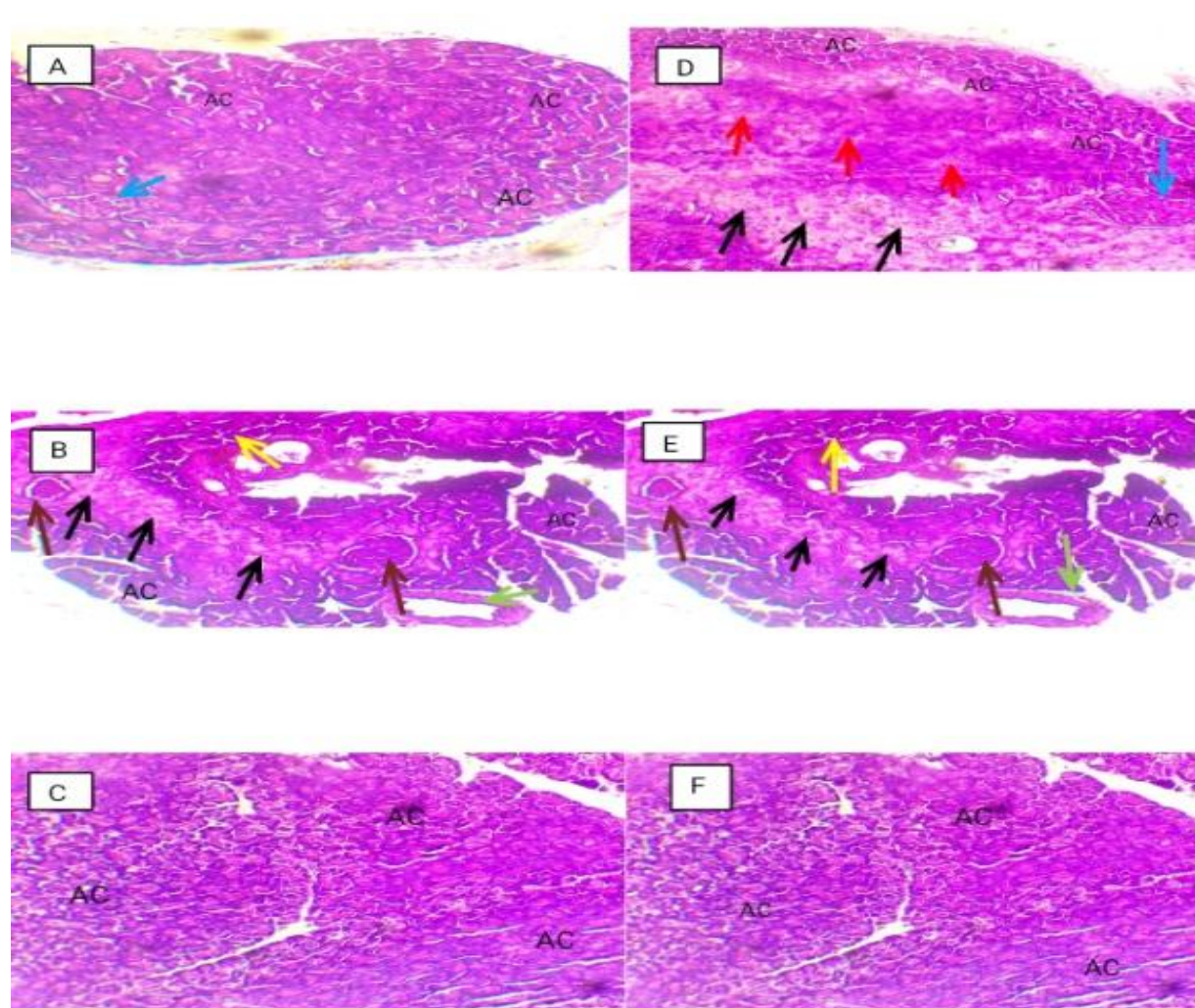


Fig 1. Histopathology of pancreas tissue (HandE stain at X100). (A) normal control, (B) untreated control, (C) glibenclamide, (D) 100mg/kg, (E) 200mg/kg, (F) 400mg/kg

DISCUSSION

The present study evaluated the antihyperglycemic, hepatoprotective, hypolipidemic, haematological, and histopathological effects of *Balanites aegyptiaca* aqueous fruit pulp extract in streptozotocin (STZ) induced diabetic rats.

At baseline, all diabetic groups had markedly elevated blood glucose compared to the normal

control, which is expected because STZ causes partial destruction of pancreatic β -cells while nicotinamide offers only partial protection, mimicking type 2 diabetes (Zaky *et al.*, 2022). Untreated diabetic rats maintained high glucose throughout the study, while extract-treated groups showed a dose-dependent reduction, with 400 mg/kg producing values close to glibenclamide. This

glucose-lowering effect may be due to stimulation of residual β -cells to secrete insulin, enhancement of insulin sensitivity, or inhibition of intestinal glucose absorption, as earlier reported for *Balanites aegyptiaca* by Gamde *et al.* (2023).

Body weight decreased progressively in untreated diabetic rats, which is a common feature of uncontrolled diabetes due to increased protein catabolism and depletion of fat stores for energy (American Diabetes Association, 2022). Extract treatment, particularly at higher doses, improved weight, likely because of better glycemic control and improved nutrient utilization, similar to observations by Mhya *et al.* (2018).

The untreated diabetic group had significantly higher bilirubin, AST, and ALT, and reduced albumin and total protein. In diabetes, oxidative stress and lipid peroxidation can damage hepatocytes, causing leakage of enzymes (AST, ALT) into the bloodstream (Mariam *et al.*, 2013). Elevated bilirubin may result from impaired hepatic clearance, while low albumin and protein indicate reduced protein synthesis in damaged liver cells (Bhardwaj *et al.*, 2024).

Treatment with *Balanites aegyptiaca* extract reversed these changes, suggesting hepatoprotective effects. The improvement could be due to the plant's flavonoids, saponins, and phenolics, which possess antioxidant properties that stabilize hepatocyte membranes and enhance protein synthesis (Zaky *et al.*, 2022). The 400 mg/kg dose produced values closest to normal, supporting a dose-dependent effect.

The untreated diabetic rats had high cholesterol, triglycerides, and LDL, with low HDL. This dyslipidemia is typical in diabetes because insulin deficiency/resistance increases lipolysis, raising free fatty acids, which the liver converts to triglycerides and VLDL; VLDL is then converted to LDL (ADA, 2022). Low HDL occurs because insulin normally stimulates apolipoprotein A1 synthesis; when insulin action is impaired, HDL production decreases (Bhardwaj *et al.*, 2024).

Extract treatment reduced cholesterol, triglycerides, and LDL while increasing HDL in a dose-dependent manner. This may be due to improved insulin action, decreased hepatic cholesterol synthesis, and enhanced reverse cholesterol transport, as reported by Gamde *et al.* (2023). The hypolipidemic effect

also reflects the antioxidant and metabolic-regulating properties of *Balanites aegyptiaca*.

The diabetic control group showed low RBC, Hb, and HCT, which can result from increased non-enzymatic glycosylation of RBC membranes, oxidative damage to red cells, and reduced erythropoiesis (Zaky *et al.*, 2022). Extract treatment improved these parameters, suggesting enhanced erythropoietic activity and reduced RBC destruction, likely through antioxidant protection of red cell membranes. WBC and MCV changes were less pronounced, but trends toward normalization were seen at higher doses.

Histological examination of the pancreas in untreated diabetic rats revealed vacuolation, degeneration, and atrophy of islets of Langerhans, consistent with STZ-induced β -cell injury (Gamde *et al.*, 2023). In contrast, extract-treated groups especially 400 mg/kg showed preservation of islet architecture, reduced vacuolation, and evidence of β -cell regeneration. These findings support the biochemical results and suggest that *Balanites aegyptiaca* offers structural protection to pancreatic tissue, likely via antioxidant and anti-inflammatory mechanisms.

CONCLUSION

The results indicate that *Balanites aegyptiaca* aqueous fruit pulp extract exerts antihyperglycemic, hepatoprotective, hypolipidemic, and haematoprotective effects in diabetic rats, with histological evidence supporting tissue protection and regeneration. The dose-dependent trends, with the highest efficacy at 400 mg/kg, align with previous reports and suggest that the plant could be a promising, low-cost adjunct therapy for diabetes management in resource-limited settings.

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