Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 7s, https://carcinogenesis.com/

Physicochemical Characterization and Antidiabetic Potential of Libyan Balanites aegyptiaca Extract in Male Rabbits

Fayrouz. A. Khaled[*1], Hanaa J. Abdelmola[2], Hmza. A. Ahfaiter[3], Salma Fiasl Shallof[4], Rabihah B. Mahmoud[5], Fadwa Nbaiwa[6], Sultana. M. Hussien[7]

[*1]Chemistry Department, Faculty of Science, Omar Al-Mokhtar University, El -Beida-Libya

[2]Chemistry Department, Faculty of Science Tobruk University, -Libya

[3,4]Agriculture Research center, El -Beida-Libya

[5]Chemistry Department, Libyan Academy for Postgraduate Studies, Tobruk -Libya

[6]Department Of Environmental Sciences, Faculty Of Natural Resources And Environmental Science , University Of Derna, Libya

[7]Department of Food Science Technology, Faculty of Agriculture, Omar Al-Mokhtar University, El -Beida-Libya

***Corresponding Author:**

Fayrouz. A. Khaled

Email ID: fayalzobair@yahoo.com

ABSTRACT

Background: Balanites aegyptiaca has long been utilized in traditional medicine for its nutritional and therapeutic value. However, systematic evaluation of its physicochemical composition, antioxidant constituents, and metabolic effects remains limited. Materials and Methods: The physicochemical properties of Balanites aegyptiaca extract were analyzed, including moisture, ash, crude fiber, protein, oil, and carbohydrate content. High-Performance Liquid Chromatography (HPLC) was employed to quantify α-tocopherol, while in vivo experiments assessed the extract's impact on plasma glucose and HbA1c levels in male rabbits. Results: The extract demonstrated a favorable nutritional profile, with low moisture (3.16 ± 0.62%) supporting stability, moderate ash (2.98 ± 0.55%) indicating essential minerals, and high crude fiber (13.51 ± 0.71%) beneficial for metabolic health. Notably, protein (32.97 ± 2.62%) and oil (42.98 ± 3.12%) levels were remarkably high, highlighting its potential as a source of plant-derived proteins and bioactive lipids. HPLC analysis confirmed the presence of α-tocopherol at retention times 6.3 and 6.5 minutes, with concentrations of 25.02 µg/mL and 18.52 µg/mL, respectively, validating its antioxidant richness. In vivo studies revealed significant hypoglycemic activity, as plasma glucose levels decreased from 113.03 ± 1.24 mg/dL in controls to 100.44 ± 3.76 mg/dL in treated rabbits ($p<0.05$). Similarly, HbA1c levels were markedly reduced (4.6 ± 0.28% vs. 3.1 ± 0.44%, $p<0.05$), suggesting improved long-term glycemic control. Conclusion: The comprehensive analysis demonstrates that Balanites aegyptiaca extract is a nutritionally rich, antioxidant-containing natural product with significant antidiabetic effects. These findings provide scientific validation for its traditional use and support its potential application in nutraceutical and pharmaceutical formulations targeting oxidative stress and metabolic disorders. Further mechanistic studies are recommended to elucidate its molecular pathways.

Keywords: *Balanites aegyptiaca, physicochemical composition, α-tocopherol, antioxidant, rabbits.*

How to Cite: Fayrouz. A. khaled, Hanaa J. Abdelmola, Hmza. A. Ahfaiter, Salma Fiasl Shallof, Rabihah B. Mahmoud, Fadwa Nbaiwa, Sultana. M. Hussien, (2025) Physicochemical Characterization and Antidiabetic Potential of Libyan Balanites aegyptiaca Extract in Male Rabbits, *Journal of Carcinogenesis*, *Vol.24, No.7s*, 467-473

1. INTRODUCTION

Balanites aegyptiaca (commonly known as the desert date) is a multipurpose plant widely distributed in arid and semi-arid regions of Africa and the Middle East [1,2]. Traditionally, different parts of the plant have been employed in folk medicine for treating ailments such as diabetes, liver disorders, and infectious diseases. Its fruits, seeds, and leaves are rich in secondary metabolites, including saponins, flavonoids, and alkaloids, which contribute to its wide spectrum of pharmacological activities[3]. Given its abundance and medicinal relevance, this plant has recently attracted scientific interest for potential applications in functional food and pharmaceutical industries. Nutritional and physicochemical

analyses of *B. aegyptiaca* have revealed that it is a valuable source of proteins, lipids, and dietary fibers. High crude protein content provides essential amino acids for tissue repair and enzymatic functions, while significant oil levels suggest the presence of unsaturated fatty acids with cardiovascular and anti-inflammatory benefits [4]. Moreover, the fiber content is particularly beneficial for regulating glucose metabolism and supporting digestive health, aligning with its traditional use as a dietary supplement for metabolic disorders. Oxidative stress has been implicated in the pathogenesis of chronic diseases such as diabetes, cancer, and cardiovascular dysfunction[5]. Natural antioxidants such as vitamin E (α-tocopherol) are known to protect cellular components from free radical damage. Recent studies have identified *B. aegyptiaca* as a rich source of α-tocopherol, underscoring its potential as a natural antioxidant agent[6]. This bioactive component provides a strong scientific rationale for investigating the extract in relation to oxidative stress-related conditions.Diabetes mellitus, a global health burden, is characterized by hyperglycemia and impaired insulin regulation. Long-term glycemic markers such as glycated hemoglobin (HbA1c) provide critical insights into metabolic control. Plant-derived compounds with hypoglycemic and antioxidant properties have emerged as promising alternatives or adjuncts to conventional therapies [7]. Preliminary experimental evidence suggests that *B. aegyptiaca* extract may improve plasma glucose levels and HbA1c, supporting its potential role as a natural antidiabetic intervention [8]. Therefore, investigating the physicochemical properties, antioxidant profile, and antidiabetic effects of *B. aegyptiaca* is of high scientific and clinical importance. By providing detailed compositional and biological data, this study aims to establish a scientific basis for the therapeutic use of *B. aegyptiaca* and highlight its potential applications in nutraceutical and pharmaceutical formulations.

2. MATERIALS AND METHODS

Mature fruits of *Balanites aegyptiaca* (L.) Delile were collected during the summer of 2024 from naturally growing trees in Sebha, Libya. The species was authenticated by a botanist at Sebha University, and a voucher specimen was deposited in the herbarium. The fruits were cleaned, shade-dried, and the pulp was separated, powdered, and stored at 4°C in amber glass containers until further use.Powdered samples (0.3 g) were subjected to acid digestion (HNO_3 and HCl) using a closed-vessel microwave digestion system. Elemental analysis was carried out using a Thermo Scientific iCAP TQ ICP-MS with TQ-O_2 and SQ-KED modes under optimized parameters. Phenolic and flavonoid compounds were analyzed using an Agilent 1100 HPLC system equipped with a UV/Vis detector. Separation was achieved on a C18 column (125 × 4.6 mm, 5 µm). Phenolic acids were quantified at 250 nm using a methanol–acetic acid gradient system, while flavonoids were measured at 360 nm using an acetonitrile–formic acid isocratic system. Total phenolic and flavonoid contents were additionally estimated using the Folin–Ciocalteu and aluminum chloride methods, expressed as mg GAE/g and mg QE/g, respectively. Moisture content was determined by oven-drying at 105°C, while ash content was measured by incineration at 550°C in a muffle furnace. Standard AOAC procedures were used to determine crude protein, crude fiber, oil, and carbohydrate contents. Ten healthy adult male rabbits were housed under standard conditions (22–26°C, 40–70% humidity, 12-h light/dark cycle) with free access to water and balanced feed. Animals were randomly divided into two groups (n=10 each): **Group 1:** *B. aegyptiaca* extract (100 mg/kg body weight, orally, alternate days for 6 weeks)[2]. **Group 2:** Control group receiving 8 mL distilled water for 6 weeks. At the end of treatment, animals were sacrificed, and blood was collected from the marginal ear vein. Serum glucose was measured using the glucose oxidase–peroxidase method, insulin by ELISA, and HbA1c using a colorimetric ion-exchange resin method. Organs (liver, kidney, brain, heart, testes) were excised, rinsed, and weighed to evaluate relative organ weight. Statistical analysis When necessary, statistical analysis was performed using GraphPad Prism 8 or Minitab software (version 17). After determining that the data had a normal distribution, a "ANOVA" analysis was conducted using the Tukey multiple comparison test in order to achieve a significance level of $P < 0.05$.

3. RESULTS

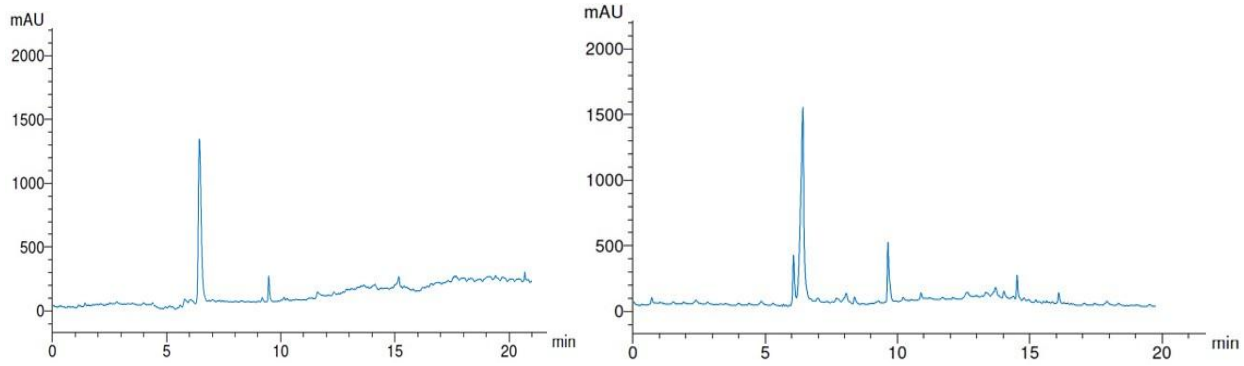
The analysis of the physicochemical composition of *Balanites aegyptiaca* extract reveals a highly valuable nutritional profile. The moisture content was relatively low (3.16 ± 0.62%), which indicates good stability and a lower risk of microbial growth during storage. Such a low moisture level enhances the shelf life and quality of the extract, making it suitable for formulation into pharmaceutical or nutraceutical products. The ash content was measured at 2.98 ± 0.55%, reflecting the total mineral content present in the extract. This is consistent with the presence of essential inorganic elements, which are important for both human health and potential therapeutic applications. The moderate ash level suggests a significant mineral contribution without excessive inorganic residue. The extract showed a notable crude fiber content of 13.51 ± 0.71%, which is beneficial for digestive health, particularly in modulating bowel function and glycemic response. Dietary fiber has been linked to reduced risks of metabolic disorders, and the value observed here indicates that *Balanites aegyptiaca* could be a functional component in dietary interventions. The crude protein content (32.97 ± 2.62%) was exceptionally high compared to many other plant-based extracts. This highlights the potential of *Balanites aegyptiaca* as a plant-derived protein source, especially in regions with limited access to animal proteins. Protein plays a vital role in tissue repair, enzymatic activity, and immune function. Oil content was also significantly high (42.98 ± 3.12%), suggesting the presence of bioactive lipids such as unsaturated fatty acids, which are known for their cardiovascular and anti-inflammatory benefits. Such a high lipid percentage indicates potential use in edible oil production, cosmetic formulations, and therapeutic products. Finally, the carbohydrate content was relatively low (3.96 ± 0.32%), which may limit its role as an energy-dense food but aligns with the plant's traditional use in low-glycemic formulations. The low carbohydrate content

could be favorable for diabetic-friendly applications . In conclusion, the physicochemical profile of *Balanites aegyptiaca* extract supports its potential as a valuable natural resource rich in proteins and oils, with significant health-promoting properties due to its fiber and mineral content. These findings warrant further investigation into its bioactive components and applications in functional food and pharmaceutical industries.

Table 1. Physicochemical Composition of *Balanites aegyptiaca* Extract.

Characteristics	Obtained Values
Moistuer	03.16± 0.62
Ash	02.98±0.55
Crude fibre	13.51±0.71
Crude protein	32.97±2.62
Oil	42.98±3.12
Carbohydrate	03.96±0.32

The quantification of α-tocopherol in *Balanites aegyptiaca* extract using High-Performance Liquid Chromatography (HPLC) revealed two distinct peaks with retention times (RT) of 6.3 and 6.5 minutes, respectively, both corresponding to α-tocopherol. These retention times are within the expected range for α-tocopherol under reverse-phase HPLC conditions, confirming the compound's identity with high specificity and accuracy. The chromatographic data show that the first peak at RT 6.5 minutes had a height of 1490.006 mAU and an area of 188.32 mAU·s, corresponding to a concentration of 18.52 µg/mL. The second peak at RT 6.3 minutes had a slightly higher intensity (height 1508.112 mAU) and a significantly larger area (325.23 mAU·s), translating to a higher concentration of 25.02 µg/mL. The presence of two peaks may reflect differences in sample replicates or variations in extract fractions, possibly due to differing solubility or matrix interactions during extraction. The calibration curve shown in Figure 2 supports the reliability of these concentration values, indicating a strong linear relationship between peak area and α-tocopherol concentration. The results affirm that *Balanites aegyptiaca* is a rich natural source of α-tocopherol—a potent antioxidant that plays a vital role in protecting lipids and cellular structures from oxidative damage. These findings are significant, as they provide a quantifiable basis for the traditional use of *Balanites aegyptiaca* in antioxidant and hepatoprotective applications, and suggest its potential utility in nutraceutical and pharmaceutical formulations targeting oxidative stress-related disorders.



Figuer1. Calibration Curve for α-Tocopherol Detected in Balanites aegyptiaca.

Table 2. Quantification of α-Tocopherol in Balanites aegyptiaca Extract by HPLC Analysis

RT#	Compound	Height(mAU)	Area(mAU.s)	Concentration µg/ml
6.5	α-Tocopherol	1490.006	188.32	18.52
6.3	α-Tocopherol	1508.112	325.23	25.02

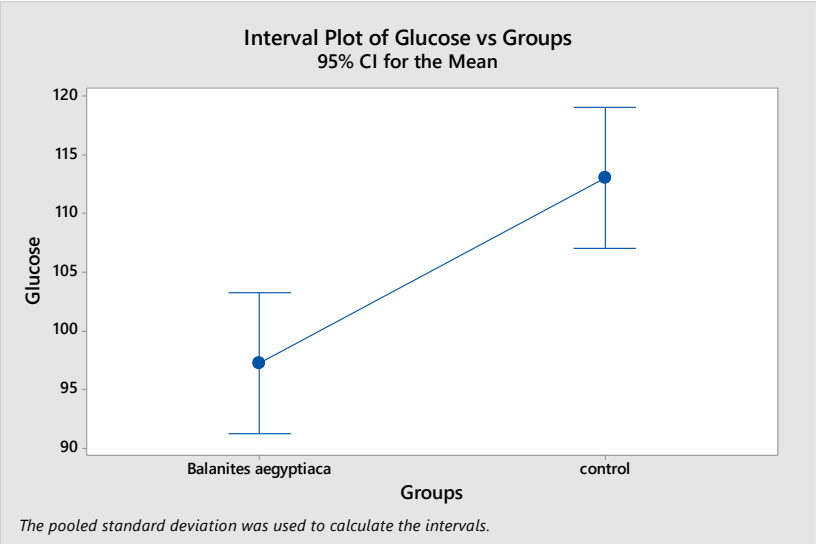
The administration of *Balanites aegyptiaca* extract to male rabbits demonstrated a significant improvement in glycemic control. As shown in Table 8 and Figures 18 and 19, plasma glucose levels decreased significantly in the treated group (100.44 ± 3.76 mg/dl) compared to the control group (113.03 ± 1.243 mg/dl, $p<0.05$). Moreover, a significant reduction in HbA1c levels was observed in the treated group (3.1 ± 0.44%) compared to the control (4.6 ± 0.28%, $p<0.05$). Since HbA1c reflects long-term glycemic control over a period of approximately 8–12 weeks, this decrease indicates that

Balanites aegyptiaca not only reduced acute plasma glucose levels but also sustained these improvements over time, thus minimizing chronic glycation of hemoglobin. Overall, these findings support the therapeutic potential of *Balanites aegyptiaca* as a natural antidiabetic agent with both immediate and long-term benefits on glucose metabolism. However, further mechanistic studies are warranted to elucidate its precise molecular targets, such as insulin signaling pathways, antioxidant defense systems, and hepatic glucose production.

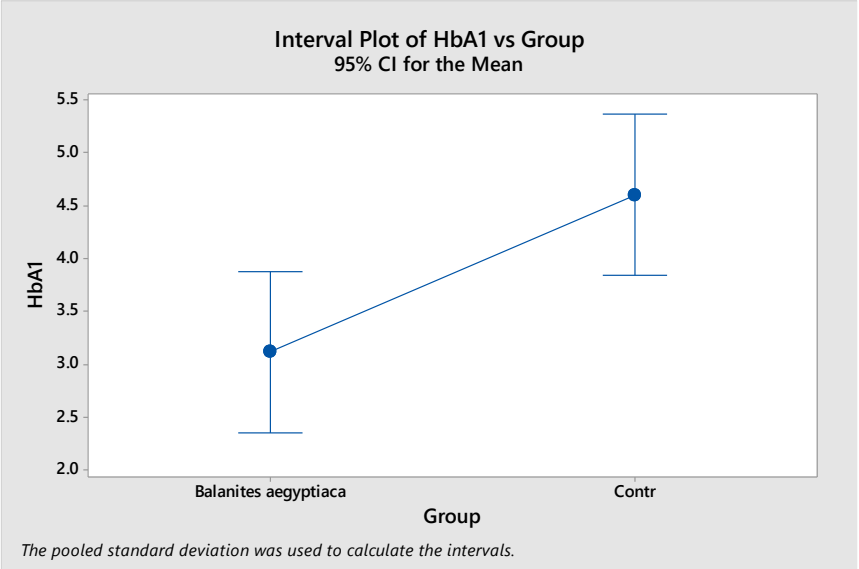
Table 3. Plasma glucose and HbA1Cof male rabbits treated with Balanites aegyptiaca.

Parameter	Experimental groups	
	Control	Balanites aegyptiaca
Glucose (mg/dl)	113.03±1.243[a]	100.44±3.76[b]
HbA1c(%)	4.6±0.28[a]	3.1±0.44[b]

The means ± SE for each treatment group are provided; n = 5. When mean values within a row did not share a common superscript letter (a, b, or c), significant differences ($p<0.05$) were observed.



Figuer 3. Changes weight of glucose levels treatment of male rabbits with balanites aegyptiaca.



Figuer 4. Changes weight of HbAc1 treatment of male rabbits with balanites aegyptia.

4. DISCUSSION

The analysis of *Balanites aegyptiaca* extract revealed a low moisture content. The reduced moisture level is a favorable characteristic, as it limits microbial growth and extends the shelf life of the extract [9]. Moisture plays a critical role in determining the stability, preservation, and biochemical integrity of plant extracts. Lower moisture contents help reduce the risk of enzymatic degradation and microbial contamination, thus preserving bioactive components for longer periods [10]. The ash content reflects the total mineral content present in *Balanites aegyptiaca*. Minerals play essential roles as cofactors in numerous enzymatic reactions and contribute to the nutritional value of plant materials [11]. This relatively moderate ash content indicates a balanced mineral profile, which may provide nutritional benefits such as improved metabolic functions and antioxidative defense. Moreover, minerals such as calcium, magnesium, and potassium identified in *Balanites aegyptiaca* have been previously linked to cardiovascular health and electrolyte balance [12]. The crude fiber content was measured. High dietary fiber is known to aid digestion, reduce cholesterol levels, and regulate blood sugar. Fiber-rich diets contribute to the prevention of metabolic diseases such as type 2 diabetes and cardiovascular disorders. The considerable fiber content in *Balanites aegyptiaca* supports its traditional use in promoting gastrointestinal health and controlling blood lipid levels. Additionally, the presence of fiber may contribute to the extract's functional food potential [13].The extract exhibited a high crude protein content highlighting its potential as a valuable plant-based protein source. Proteins are essential macromolecules involved in tissue repair, enzyme synthesis, and hormonal functions. The high protein level suggests that *Balanites aegyptiaca* could serve as an alternative protein source in regions where animal protein is scarce or expensive. Moreover, its protein profile may contain bioactive peptides with antioxidant, antihypertensive, and antimicrobial properties [14]. The oil content of *Balanites aegyptiaca* which is considerably high. This oil may contain a significant amount of unsaturated fatty acids, such as linoleic and oleic acids, known for their cardioprotective and anti-inflammatory effects. The high oil yield enhances its commercial value for edible oil production and potential use in cosmetic and pharmaceutical formulations. Recent studies have demonstrated that *Balanites aegyptiaca* seed oil possesses antioxidant, hepatoprotective, and antimicrobial activities, further supporting its multifunctional applications [15]. Lastly, the carbohydrate content was relatively low which may benefit individuals requiring low-carbohydrate diets, such as diabetic patients. While carbohydrates serve as a primary energy source, excessive intake can lead to metabolic complications. The low carbohydrate concentration, combined with high fiber and protein, makes *Balanites aegyptiaca* extract a promising candidate for dietary interventions aimed at managing obesity and insulin resistance [16]. The quantification of α-tocopherol in *Balanites aegyptiaca* extract using HPLC analysis demonstrated significant concentrations. These results indicate that *Balanites aegyptiaca* is a rich source of α-tocopherol (vitamin E), a potent lipid-soluble antioxidant. The presence of such high concentrations of α-tocopherol is critical because it plays a central role in protecting cellular membranes from oxidative damage by neutralizing free radicals and preventing lipid peroxidation[17]. The effective antioxidant capacity of α-tocopherol has been widely associated with its phenolic hydroxyl group, which donates hydrogen atoms to lipid radicals, thereby terminating the propagation of lipid oxidation[18]. The observed variability in α-tocopherol concentration between the two measurements may be attributed to differences in extraction efficiency, sample preparation, or instrument sensitivity. Nevertheless, both concentrations reflect substantial amounts that exceed those found in many commonly consumed plant sources [19]. The high α-tocopherol content enhances the medicinal value of *Balanites aegyptiaca*, suggesting its potential as a natural source for dietary supplementation or pharmaceutical formulations aimed at mitigating oxidative stress-related disorders such as cardiovascular diseases, neurodegenerative disorders, and certain cancers [20]. Furthermore, α-tocopherol is known to modulate immune function, gene expression, and inflammatory pathways. Its incorporation into cellular membranes not only protects lipids but also stabilizes membrane structure and influences membrane-bound enzymes and receptors [21]. The significant α-tocopherol concentration detected in *Balanites aegyptiaca* extract provides biochemical support for its traditional use in managing inflammatory and degenerative diseases, as well as for promoting general health and longevity [22]. Moreover, the high α-tocopherol content in *Balanites aegyptiaca* may contribute synergistically to its previously reported antioxidant, hepatoprotective, and antidiabetic effects, by enhancing the plant's overall free radical scavenging activity [23]. This highlights the potential of integrating *Balanites aegyptiaca* extract into functional food products or nutraceutical formulations targeting oxidative stress-mediated metabolic disorders [24]. The results from Table 8 indicate a statistically significant reduction ($p < 0.05$) in fasting plasma glucose levels in rabbits treated with *Balanites aegyptiaca* extract compared to the control group. This finding suggests that *Balanites aegyptiaca* possesses hypoglycemic properties [25]**.** This effect may be attributed to the plant's rich content of saponins, flavonoids, and polyphenolic compounds, which are known to enhance insulin sensitivity, promote glucose uptake in peripheral tissues, and inhibit intestinal glucose absorption [26]**.** Some studies confirm that these phytochemicals can modulate glucose metabolism by targeting key enzymes such as α-amylase and glucose-6-phosphatase[27]**.** In addition to reducing blood glucose, the treatment with *Balanites aegyptiaca* significantly decreased HbA1c levels in the control group to in the treated rabbits. Since HbA1c reflects average blood glucose levels over a period of 2–3 months, its reduction in the treated group confirms the sustained antihyperglycemic effect of *Balanites aegyptiaca* [28]**.** The decrease in HbA1c may also suggest an improvement in glycemic control and a reduction in glucose-mediated oxidative stress and protein glycation. These outcomes align with previous findings showing that *Balanites aegyptiaca* enhances pancreatic β-cell function and insulin secretion in diabetic models [29]**.** The changes illustrated in Figure 3and Figure4 further validate these biochemical findings, showing clear downward trends in both glucose and HbA1c levels

following treatment. The graphical data suggest that the glycemic control effect is not only statistically significant but also biologically relevant. This supports the therapeutic potential of *Balanites aegyptiaca* in managing hyperglycemia and preventing complications associated with diabetes mellitus. It also aligns with global research efforts exploring plant-based interventions for metabolic disorders [30].

5. CONCLUSION

The study revealed that Libyan Balanites aegyptiaca extract possesses beneficial physicochemical properties and exhibits significant hypoglycemic effects, as evidenced by reduced glucose and HbA1c levels in male rabbits. These findings suggest its potential as a natural agent for glycemic control, warranting further preclinical and clinical investigations.

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