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***Balanites aegyptiaca* (Heglig Dates) Reduces Oxidative Stress, and Biophysical Alterations of Erythrocyte Membranes in Streptozotocin-Induced Diabetic Rats**

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ABSTRACT

Objectives: In diabetic conditions, hyperglycemia-induced oxidative stress promotes alterations of function and other properties of the erythrocyte membrane. In this study, we investigated the effects of *Balanites aegyptiaca* (100 mg/kg) on biophysical characteristics of erythrocyte membranes in streptozotocin-induced diabetic rats. **Methods:** Twenty-four male rats were divided into three groups as follows: Group 1, control group; Group 2, diabetic group; and Group 3, diabetic plus *Balanites aegyptiaca* (Heglig dates). After 30 days of treatment, we determined erythrocyte osmotic fragility, AC conductivity, auto-oxidation rate of hemoglobin, and electron paramagnetic resonance. **Results:** The osmotic fragility was significantly higher in the diabetic group compared to the control and diabetic with Heglig dates. We also found a significant decrease in the hemoglobin concentration. **Conclusion:** Four weeks of daily supplementation with 100 mg/kg Heglig dates suggest the beneficial role of *Balanites aegyptiaca* fruit as a hypoglycemic and could reduce the levels of oxidative stress markers by inhibiting lipid peroxidation in streptozotocin-induced diabetic rats.

Keywords: *Balanites aegyptiaca*; Erythrocytes; Electrical conductivity; Osmotic fragility; EPR signals; Diabetic

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by high blood sugar levels (hyperglycemia) resulting from defects in insulin production, resistance to insulin effect, or both^{1,2}. Persistent hyperglycemia in diabetes produces an increase in free radical generation, particularly reactive oxygen species, in all tissues due to glucose auto-

oxidation and protein glycosylation^{3,4,5}. Free radicals are produced as a by-product of normal cellular metabolism; however, the balance between ROS formation and cellular defense mechanisms has been documented to be disrupted by several conditions. Diabetes causes lipid profile abnormalities, especially higher exposure to lipid peroxidation⁶, which leads to an increased risk of atherosclerosis⁷, a significant consequence of diabetes mellitus⁸. Hemolysis of

erythrocytes mechanism following oxidative stress in vivo and in vitro⁹. Haemoglobin continues to be the major source of damage when different oxidative drugs are administered¹⁰. lipid peroxidation, a reduced antioxidant status, and higher free radical generation have been found in these individuals, demonstrating increased oxidative stress^{11,12}. Free radicals may have a major role in the causation and complications of diabetes¹³. In diabetes, alterations in the endogenous free radical scavenging defense mechanisms may result in inadequate scavenging of reactive free radicals, resulting in oxidative damage and injury.

Balanites aegyptiaca fruit flesh can be obtained from palm trees that grow in the desert of the southern valley of Egypt (Halaieb and Shelateen area). *Balanites aegyptiaca* has a wide ecological distribution and it belongs to the family Balanitiaceae and is also known as Hegleg or Balah El-Abeed. The date is dark brown in color, and the fleshy pulp of both unripe and ripe fruits is edible and eaten dried or fresh. It contains protein, lipid, carbohydrate, alkaloid, saponin, flavonoid, and organic acid.

Streptozotocin (STZ) is commonly used to produce experimental T1DM, and its diabetic effect is due to its extremely specialized cytotoxic activity on β cells, which results in an increase in the generation of oxygen free radicals which lead to oxidative stress^{14,15}. *Balanites aegyptiaca* is a widely distributed African plant of medicinal interest. In Egyptian folk medicine, its fruit mesocarp is broadly applied as an oral antidiabetic drug^{16,17}.¹⁸ reported that the diabetic group recorded hyperglycemia, hypoinsulinemia, a significant increase in many parameters of liver and kidney functions markers, alterations in proteins level, and decreased liver glycogen content. While treatments with *Balanites aegyptiaca* (seeds) were ameliorated most of the harmful effects of alloxan (chemical components induced diabetes) and demonstrated partial improvement in histological changes produced by alloxan. *Balanites aegyptiaca* (seeds) extraction has hypoglycemic, hypolipidemic effects, improving insulin level and reducing insulin resistance. According to¹⁹, erythrocyte count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular hemoglobin concentration were decreased in diabetic rats. C-peptide and insulin levels were significantly decreased in STZ-induced diabetic rats due to the destruction of β -cells of the pancreas that will restrict insulin release. Oral treatment of Heglig dates dramatically enhanced plasma insulin and C-peptide levels in STZ-induced diabetic rats when compared with diabetic control rats. This is because flavonoids found in balanites stimulate the secretion of insulin from β -cells of the pancreas²⁰.

MATERIAL AND METHODS

Chemicals

Streptozotocin solution was prepared with 1 g streptozotocin powder was dissolved in 100 mL 0.9 N NaCl saline solution to make streptozotocin solution. Streptozotocin solution was injected intraperitoneally at a dose of 47 mg/kg of body weight in rats.²¹

Experimental animals

All animals are housed in specially built cages with proper ventilation, temperature, and humidity for seven days after transfer. They were kept on standard food pellets containing all nutritive elements and water *ad libitum*.

Experimental design

Twenty-four adult male rats' average weight (180-200 g) were performed in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. Animals were divided equally into 3 groups; each with eight rats: control, diabetic, and rats treated orally with *Balanites aegyptiaca* extract (Heglig dates) 100 mg/kg (2 ml) for 30 days.

Induction of diabetes

Diabetes mellitus was induced by injection of streptozotocin in a single dose at (47 mg/kg) for 30 days diluted in saline solution for all animals except eight rats, which will be the negative control group²¹.

Extraction of the fruit flesh

Fruit flesh was sliced and weighed, and the seeds were discarded. The flesh was dried for one hour at 110 degrees Celsius, then the temperature was decreased to 70°C for 48 hrs. Fruit flesh was extracted with water, in a Soxhlet apparatus for 10 hours according to the Association of Official Analytical Chemists (AOAC, 1970) procedure.

Blood Samples collection

Ten μ L of whole fresh blood was incubated in 5 mL normal saline. After 30 min, the samples were centrifuged at 3,000 rpm for 10 min, and then the supernatant was removed to obtain the packed cells. They were lyophilized using a freeze dryer at 60 °C and 70 mbar.

Registration of hemolysis

Hemolysis of erythrocytes in all groups was measured spectrophotometrically (Jenway model 6300) at 577nm (spin state band of iron- hem), where the decrease in the intensity of the peak at 577nm indicates the degree of haemoglobin breakdown.

Osmotic fragility

Osmotic fragilities were determined from 0.30–0.65% saline solution is used to determine osmotic fragility. Normal erythrocytes remained suspended in an isotonic solution for 60 min without rupturing. However, when they were suspended in a hypotonic solution, they absorbed the fluid, swelled, and got lysed. This is known as osmotic fragility. The hypotonicity of RBCs undergoing lysis depends on the integrity of their membrane. Osmotic fragility excesses when the membrane becomes weak. Thus, osmotic fragility is a measure of the strength of the erythrocyte membrane.²²

AC conductivity measurement

AC conductivity measurement were carried out using LCR meter type HIOKI 3531, in the frequency range 40 kHz to 5 MHz. The measuring cell is a parallel plate with platinum electrodes²³, with an area of 2 cm² and a separating distance of 1cm. The erythrocytes samples were diluted in isotonic buffered saline (pH 7.4). The samples were incubated in a water bath at 37 °C during measurement.

The measured parameters were conductance G, from which the conductivity σ can be calculated as follows:

$$\sigma = G \frac{d}{A}$$

where A is the area of the electrode, d is the distance between the two electrodes.

Electron paramagnetic resonance (EPR)

An X-band ESR spectrometer (Bruker, EMX made in Germany) was used to measure EPR spectra of lyophilized erythrocytes at ambient temperature using a standard rectangular cavity (4102 ST) operating at 9.7GHz with a 100 kHz modulation frequency. The ESR parameters were chosen in order to achieve the maximum signal-to-noise ratio.

Statistical analysis

The values in this study are provided as mean \pm standard deviation. The significance of the difference between each value presented by different groups was calculated by the Student t-test and values with $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Registration of hemolysis

The release of haemoglobin in erythrocytes as shown in **Figure 1**, where the absorbance for each group decreases with time, indicating that the percentage of hemolysis increases over time. In the diabetes group, there was a significant increase in the auto-oxidation rate of haemoglobin (hemolysis) compared to control, while the auto-oxidation rate was decreased significantly in the rats treated orally with *Balanites aegyptiaca* when

compared to the diabetic control. These results indicate that the number of erythrocytes rapidly declines in a diabetic group (hemolysis), causing lipid peroxidation and oxidation of haemoglobin (oxidation of Fe⁺² to Fe⁺³)^{24,25,26}. Hypoinsulinaemia raises the activity of the enzyme fatty acyl-coenzyme A oxidase, which initiates β -oxidation of fatty acids, leading to lipid peroxidation²⁷. Membrane function is harmed by increased lipid peroxidation by reducing membrane fluidity and alters the activity of membrane-bound enzymes and receptors²⁸.

It is clear from **Figure 1** that the diabetes group is the strongest hemolytic agent in comparison with the control and diabetic group treated by *Balanites aegyptiaca* (100 mg/kg, *p.o.*) in which the hemolysis is fulfilled at about 20 minutes.

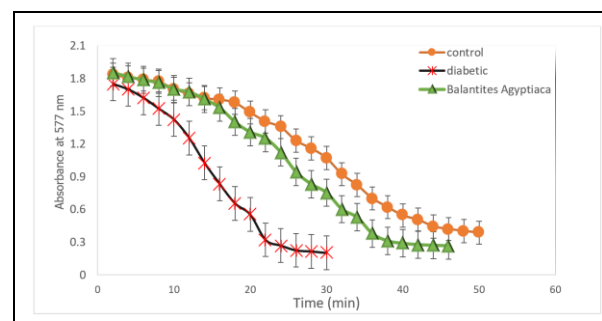


Figure 1. Effect of Heglig dates (100 mg/kg, *p.o.*) on hemolysis of erythrocytes at 577 nm in STZ-induced diabetic rats.

Erythrocyte fragility

osmotic fragility curves of the normal, diabetic, and treated rats with *Balanites aegyptiaca* groups are shown in **Figure 2**, where the % hemolysis has been plotted as a function of the percentage concentration of sodium chloride. These results show that diabetic rats treated with *Balanites aegyptiaca* had a significant reduction in erythrocyte osmotic fragility while the fragility of erythrocytes has risen in the diabetic group, which could be due to increased lipid peroxidation. Changes in membrane structure and function have been linked to lipid peroxidation. Increased lipid peroxidation has been linked to a decrease in cell fluidity and an increase in osmotic fragility²⁹.

AC conductivity measurement

The conductivity depends on the dynamical ionic transport through the membrane. It accounts for both the structural ionic and polar group arrangements and the dynamical ionic transport processes of the membrane³⁰. Variation in conductivity of erythrocytes with frequency in the range 50 Hz to 5 MHz is shown in **Figure 3** for normal, diabetic, and treated rats with *Balanites aegyptiaca* groups.

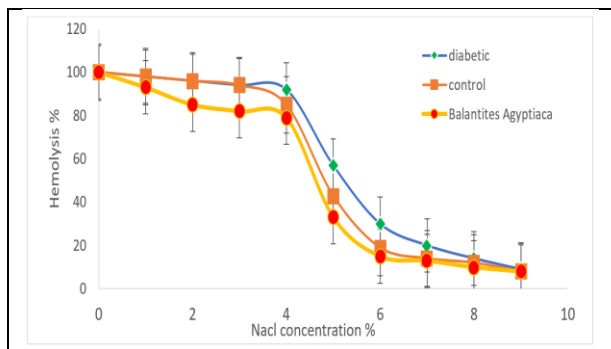


Figure 2. Percentage of osmotic fragility for normal, diabetic, and diabetic group treated by *Balanites aegyptiaca* (100 mg/kg, p.o.) of erythrocytes against sodium chloride at various concentrations.

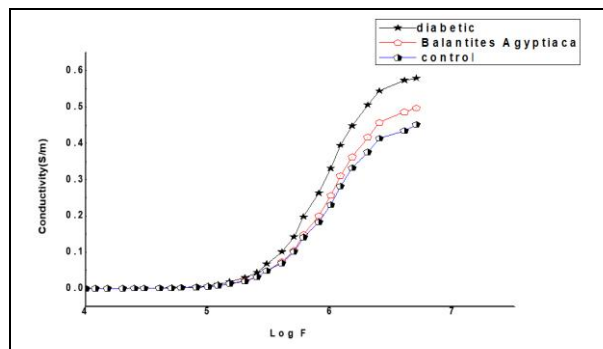


Figure 3. AC electrical conductivity for normal, diabetic, and diabetic group treated by *Balanites aegyptiaca* (100 mg/kg, p.o.) of erythrocytes.

Electron paramagnetic resonance (EPR)

Table 1 shows the calculated spin number of control, diabetic, and diabetic group treated by *Balanites aegyptiaca* erythrocytes.

Table 1. Calculated spin number of control, diabetic, and diabetic group treated by *Balanites aegyptiaca* erythrocytes.

Groups	Statistics	SPIN No
Control	x̄	4.550 x10 ¹⁵
	SD	6.11 x10 ¹⁴
<i>Balanites aegyptiaca</i>	x̄	4.89 x10 ¹⁵
	SD	6.15 x10 ¹⁴
	P (normal group)	0.078
	P (diabetic group)	0.002
Diabetic	x̄	5.94x10 ¹⁵
	SD	1.43 x10 ¹⁴
	P	0.002

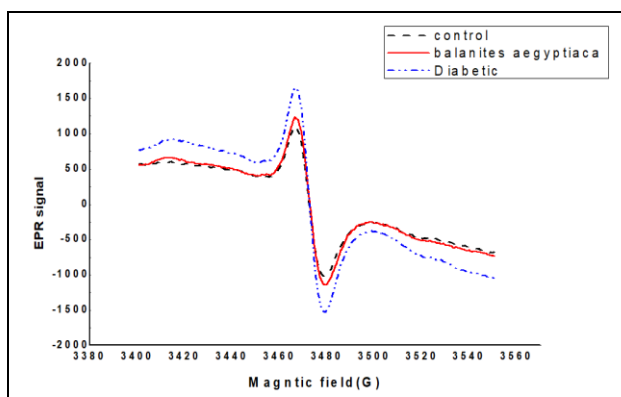


Figure 4. EPR signals for control, diabetic, and a diabetic group treated by *Balanites aegyptiaca* of erythrocytes.

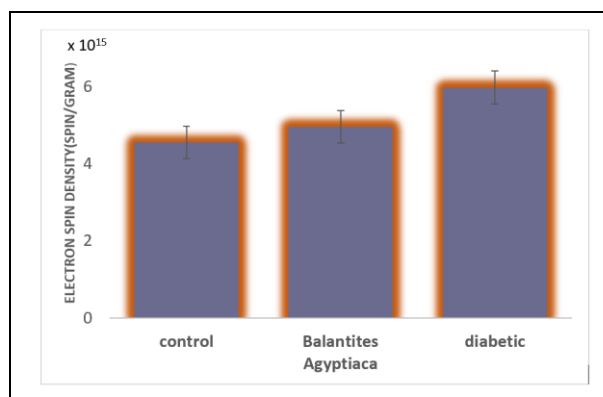


Figure 5. Changes in the EPR signal intensity for control, diabetic, and diabetic group treated by *Balanites aegyptiaca* of erythrocytes.

Figure 4 shows an example of the X-band (9 GHz) EPR spectra of the erythrocyte at ambient temperature. It is the summation of individual spectra corresponding to all free radicals simultaneously present in the sample. The figure demonstrates the increase in the EPR spectra of a diabetic group of erythrocytes compared to the control group. **Figure 5** demonstrates an increase in the spin density in the case of the diabetic group. It is clear that diabetes caused an increase in free radical density by 76.5% compared to the normal group.

CONCLUSION

The obtained results suggest that there is an increase in electrical properties (ac conductivity), hemolysis percentage, and number of spin density of diabetic erythrocytes compared to healthy individuals. Also, suggest the beneficial role of *Balanites aegyptiaca* (100 mg/kg) fruit as a hypoglycemic and could reduce the levels of oxidative stress markers by inhibiting lipid peroxidation in streptozotocin-induced diabetic rats.

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Conflict of interest

The author declares that there isn't any conflict of interest regarding the publication of this paper.

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