Antidiabetic Activity of Standardized Extracts of *Balanites aegyptiaca* Fruits using Cell-based Bioassays

Amira Abdel Motaal,1** Sherif Shaker,2 Pierre S. Haddad3

1Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Ainy St., Cairo 11562, Egypt.
2Pharmaceutical Department, Heliopolis Academy, Sekem, 3 Cairo-Belbeis Road, El Horreya, Cairo, Egypt, P.B. 2834.
3Natural Health Products and Metabolic Diseases Laboratory, Department of Pharmacology and Montreal Diabetes Research Centre, Université de Montréal, Montreal, Quebec, Canada H3 C3J7

**ABSTRACT**

The antidiabetic activities of different extracts and fractions of *Balanites aegyptiaca* were tested in cultured C2C12 skeletal muscle cells and 3T3-L1 adipocytes. An 18-h treatment with 200 µg/mL of the sugars fraction (A1) showed the highest activity as it increased basal glucose uptake by 52% in muscle cells; which is twice the activity of 100 nM insulin (insulin equivalent (IE) = 2.0 ± 0.07). The dichloromethane (E) and ethyl acetate (F) successive extracts exerted 37 and 41% increase in the glucose uptake, respectively. Only E and F accelerated the triglyceride accumulation in pre-adipocytes undergoing differentiation, comparably with 10 µM rosiglitazone (rosiglitazone equivalent (RE) was 1.6 ± 0.3 and 0.7 ± 0.1, respectively). Gas chromatography (GC) analysis of A1 revealed the presence of xylose, rhamnose, sorbitol, fructose, galactose and glucose. The active extracts E and F were standardized by high-performance liquid chromatography (HPLC) to contain 0.031 and 0.239% of rutin, 0.007 and 0.004% of isorhamnetin, respectively.

**Key words:** balanites, C2C12, diabetes, fruit, sugars, 3T3-L1.

**INTRODUCTION**

*Balanites aegyptiaca* Del. (Zygophyllaceae) is used traditionally in African countries as an anthelmintic[1] and in the treatment of jaundice.[2] The fruits are used as an oral antihyperglycemcic in Egyptian folk medicine[3] and herbalists in the Egyptian market sell the fruits as an antidiabetic agent. However, quality control of such herbal products remains a great challenge. The aqueous extract of the mesocarp of the Balanites fruits revealed significant antidiabetic activities in STZ-induced diabetic rats and mice[3,4] and several saponins were isolated from the mesocarp.[3,5-8] Nevertheless, the target tissues and mechanisms of action of this herb are not yet well understood.

The aim of the present study was to assess the antihyperglycemic activities of different extracts and fractions of *Balanites aegyptiaca* fruits defining target tissues and the corresponding group of bioactive compounds through cell-based bioassays using C2C12 myotubes and 3T3-L1 adipocytes.

**MATERIALS AND METHODS**

**Plant material**

The fruits of *Balanites aegyptiaca* were brought from Aswan and were authenticated by Prof. Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen (voucher no. 201) was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

**Extraction and fractionation**

Balanites fruits (1 kg) were defatted by n-hexane and successively extracted by maceration in each of dichloromethane, ethyl acetate, methanol and water. Extracts were filtered and concentrated under vacuum at 50 °C giving extracts E, F, G and H, respectively. Similarly, the fruit was extracted with methanol, cold water, hot water and 70% ethanol to give extracts A, B, C and D, respectively. The dried methanol extract (3 gm) was defatted with n-hexane. The mother liquor was chromatographed on Diaion HP-20 (Supelco, St. Louis, MO, USA) column and eluted with H2O, 40% methanol and 95% methanol successively, giving 31, 12.5 and 19% of free sugars and other aqueous soluble substances (A1), as well as substances that do not dissolve completely in water, like phenolics (A2)
and saponin-rich (A3) fractions, respectively.9,10 The dried extracts and fractions were dissolved in dimethyl sulfoxide (DMSO; 0.1% final concentration) for biological screening.

**HPLC analysis of active extracts**

An HPLC method was adapted for analysing *Balanites aegyptiaca* E and F extracts. An Agilent 1100 series HPLC was used, equipped with Agilent G1311A quaternary pump, a G1314A variable wavelength detector and a G1328A manual injector. *Balanites aegyptiaca* ethyl acetate and dichloromethane fractions were dissolved in methanol (17.24 and 22.34 mg/ml, respectively), filtered through PTFE 0.45 μM syringe filter (Macherey-Nagel, Germany) and injected into a Hypersil 100 RP-18, 5 μm, 250 × 4 mm column. The mobile phase used was acetonitrile (solvent A) and 1% acetic acid in water (solvent B). Gradient elution was carried out at a flow rate of 1.0 ml/min as follows: 0-20 min 30 to 75% A in B. Measurements were made with an injection volume of 20 μl and UV detection at 370 nm. Standard calibration curves were prepared using five concentrations of isorhamnetin (1, 2, 4, 8 and 10 μg/ml) and rutin (4, 8, 10, 16 and 20 μg/ml) in methanol. For each sample, three replicate assays were performed.

**GC analysis of the sugar fraction**

Fraction A1 (70 mg) was extracted with hot water and derivatised.11 The derivatised sugar solution (1 ul) was injected onto a 3 m × 250 μm capillary column packed with 14% cyanopropyl phenyl methyl (HP-1701) heated isothermally at 150 °C for 2 min, then temperature programmed at 7 °C/min to 200 °C. GC apparatus HP6890 was used and injector/detector (FID) temperature was 270 °C with nitrogen carrier gas at 40 ml/min. Sugar standards (xylose, rhamnose, sorbitol, fructose, galactose, glucose and glucuronic acid) were similarly prepared and injected to identify the retention time of each.

**Cell culture**

C2C12 myoblasts and 3T3-L1 preadipocytes (ATCC, Manassas, VA, USA) were cultured as previously described in Dulbecco’s modified Eagle’s medium.12-15 C2C12 cells were treated with 200 μg/mL of extracts and fractions. Concentrations of 50 or 100 μg/mL were used for treating the 3T3-L1 preadipocytes. Diosgenin 50 μM (Sigma-Aldrich, Germany) was used for treating both cell lines. These concentrations were based on the maximum non-toxic concentrations determined in the employed cell lines following 18-h treatment.

**Glucose transport assay**

Differentiated C2C12 cells grown in 12-well culture plates were treated with the test solutions, vehicle control (0.1% DMSO) and a positive control (400 μg/mL metformin) for 18 h. Insulin 100 nM was added to one well as a second positive control during the assay.12,14,15 Five separate experiments, each with three replicates, were performed.

**Adipogenesis assay**

3T3-L1 cells seeded in 12-well plates were treated with the test solutions or 0.1% DMSO vehicle in differentiation medium during the entire differentiation period as described before.12,14,15 Rosiglitazone 10 µM (Alexis Biochemicals, San Diego, CA, USA), dissolved in DMSO, was used as a positive control. Three separate experiments, each with three replicates, were performed.

**RESULTS AND DISCUSSION**

**HPLC finger-print chromatograms of active extracts**

Successive extracts E and F were analyzed by HPLC to develop a fingerprint chromatogram for each. The extracts were injected against available flavonoid reference compounds (rutin, isorhamnetin, quercetin, scopoletin, caffeic acid and hyperoside). Rutin and isorhamnetin were identified and used for standardization of the extracts (Figure 1). The concentrations of rutin in *Balanites aegyptiaca* F and E extracts were found to be 0.239 and 0.031% and those of isorhamnetin were 0.004 and 0.007%, respectively.

**GC analysis of the sugar fraction**

A GC chromatogram was developed as a fingerprint for the most active sugar fraction A1 (Figure 2). The relative retention times for xylose, rhamnose, sorbitol, fructose, galactose, glucose and glucuronic acid were 7.30, 8.29, 9.66, 9.80, 10.76, 11.07 and 14.25 min, respectively. Xylose, rhamnose, sorbitol, fructose, galactose and glucose were detected in the sugar fraction with area percentages of 1.35, 26.37, 1.18, 1.51, 49.22 and 13.2%, respectively, with an unknown peak at retention time 8.18 min constituting 4.96% of the total areas. A previous analysis of the glycosyl part identified within balanites fruit revealed the presence of galactose, mannose, arabinose, xylose, rhamnose and glucuronic acid.16

**Cell-based bioassays for antidiabetic activity**

The enhancement of glucose uptake in muscle cells and the acceleration of triglyceride accumulation in differentiating adipocytes (increased adipogenesis) were studied for the plant extracts and fractions. The four extracts of balanites (A, B, C and D) exhibited similar significant activities on differentiated C2C12 myotubes as shown by their activities relative to an optimal dose of insulin (100 nM; insulin equivalents, IE: values of 0.7 ± 0.03, 0.6 ± 0.02, 0.7 ± 0.02 and 0.8 ± 0.02, respectively) (Figure 3). Extraction of the defatted fruits with solvents of increasing polarity resulted in an increase in activity to become about 1.5-fold that of...
Figure 1: HPLC chromatograms of extracts E and F showing peaks of rutin (#) and isorhamnetin (*) at retention times (min) 3.5 and 10.2, respectively. Gradient elution was carried out using acetonitrile and 1.0% acetic acid in water. Peaks were detected at 370 nm.

Figure 2: GC chromatogram of fraction A1 showing peaks of xylose (1), rhamnose (2), sorbitol (3), fructose (4), galactose (5) and glucose (6) at retention times (min) 7.30, 8.29, 9.66, 9.80, 10.76 and 11.07, respectively.
insulin in successive extracts E and F (IEs of 1.5 ± 0.07 and 1.6 ± 0.04, respectively). Only these two extracts revealed a significant activity in increasing the triglyceride accumulation in differentiated 3T3-L1 adipocytes comparable to that caused by 10 µM rosiglitazone (RE = 1.6 ± 0.03 and 0.7 ± 0.1, respectively) (Figure 4). Both extracts were standardized to their phenolic content (Figure 1). Many studies have already demonstrated the antihyperglycemic activity of flavonoids using different experimental models.\(^{14,17,18}\) Fractionation of extract A on Diaion HP-20 gave fraction A1 standardized to contain monosaccharides and possessing the highest activity among all tested extracts and fractions. Indeed, it increased basal glucose uptake in skeletal muscle cells twice as much as insulin (IE = 2.0 ± 0.07) (Figures 2 and 3). Diosgenin 50 µM, the main aglycone of the saponins of Balanites, did not show any activity on the two cell lines under the experimental conditions (results are not shown). Kamel et al., (1991)\(^{19}\) reported previously that oral administration of 80 mg/kg bw of the aqueous extract of the fruit mesocarp, as well as the polysaccharide fraction (precipitated by excess of alcohol) and the supernatant (saponin-rich), revealed significant antidiabetic activities in STZ-induced diabetic mice. In another study on STZ-diabetic rats, it was suggested that the antihyperglycemic effect of balanites aqueous extract (1.5 g/kg bw) was mediated through insulinoimimetic effect as well as inhibition of intestinal α-amylase activity.\(^{31}\)

The present studies bring forth novel data showing that the reported antihyperglycemic activity of \textit{B. aegyptiaca} can also be attributed to significant insulin-like and partly glitazone-like activities in peripheral tissues. Increased muscle basal glucose uptake thus participates in the traditionally known, and \textit{in vivo} proven, antidiabetic effect of the balanites fruits. Moreover, the sugar part (A1) and the phenolic content (E and F) contribute to a large extent to this activity. On the other hand, some of the balanites bioactive fractions (E and F) may act in a manner similar to the thiazolidinedione (e.g. rosiglitazone) family by increasing insulin sensitivity in the fat tissue.\(^{36}\) Further investigations are in progress to isolate bioactive compounds from these active fractions, possessing a greater activity compared to insulin, which could hold a great promise for developing oral antihyperglycemic lead compounds.

**ACKNOWLEDGEMENTS**

Dr. A. Abdel Motaal's scientific visit to the University of Montreal to carry out the cell-based bioassays was in the context of a grant headed by Prof. P. S. Haddad and funded by the Agence Universitaire de la Francophonie (Grant number 7044ME902). Abir Nachar and Padma Madiraju are gratefully acknowledged for their technical support.

**REFERENCES**


