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Biological Effect of *Balanitesa egyptica* on Type 2 Diabetes.

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ABSTRACT

This study was to investigate the possiblility of antidiabetic activity of the ethanolic *Balanitesa egytica* extract compared to currently available antidiabetic drug gliclazide (diamicron) against diabetic complications to induce tissues injury in rats for 30 days. Animals were divided into 5 groups.Group1, control rats (not received any medication). Group 2, rats injected intraperitoneally with single dose of streptozotocin (STZ, 50 mg/kg body weight) .Groups 3 and 4were daily oral administered with ethanolic extract of *Balanitesa egytica*(1.5,3 g/ kg B.W.) after STZ injection. Group5, was orally administered with gliclazide (10 mg/kg B.W.) after STZ injection. For all groups were determined on blood glucose, total cholesterol, triglyceride HDL, LDL in the serum of control and treated groups. Liver samples were collected to investigate glycogen content. Pancareatic sample was processed for microscopic B cell. Thus treating diabetic rats with *B. aegyptiaca* ethanolic extract (1.5,3g/ kg B.W.) rats for four weeks significantly reduced their blood glucose, total cholesterol, triglyceride and LDL coupled while increase in HDL. Histopathological investigation, pancreatic tissues of diabetic rats displayed islets shrinkage, cytoplasmic vacuolation in *β*- cells with pyknotic nuclei. Most of the cytoplasm of *β*- cells *B. aegyptiaca* ethanolic extract treatment show granulated with less vacuol. Treating diabetic animals with gliclazide improved diabetic and induced alteration in most of the above studied markers. These results suggest that *Balanitesa egytica* ethanolic extract possess multi-beneficial actions in controlling diabetes and its induced consequent complications in pancreas and liver revealing promising candidate as natural antidiabetic drug usage.

Keywords: Diabetic; *β*- cells, *B. aegyptiaca*, LDL, HDL, Streptozotocin

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INTRODUCTION

Diabetes mellitus is a chronic metabolic condition that is marked by increased circulating concentrations of glucose, which is associated with the development of long-term vascular complications. There are two predominant forms, type 1 and type2. Type 1 diabetes mellitus (T1DM) is characterized by absolute insulin deficiency that results from autoimmune destruction of pancreatic islet cells, so it referred to "insulin-dependent diabetes mellitus" **[1]**. Type 2 diabetes mellitus (T2DM) is responsible for 90 to 95% of diabetes worldwide **[2]**. Hyperglycemia resulting from unregulated glucose level is widely recognized as the causal link between diabetes and diabetic complications **[3]**. It was found that hyperglycemia cause tissue damage by mechanisms involving repeated changes in cellular metabolism **[4]**. One of the key metabolic pathways as being major contributors to hyperglycemia induced cell damage, is the nonenzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) to form Advanced Glycosylated End (AGE) product **[5]**. Production of AGE interefers with cell integrity by modifying protein function or by inducing receptors mediated. At least 90 million people throughout the world suffer from *diabetes mellitus* **[6]**.Nature is an extraordinary source of antidiabetic medicines. Where, many herbal products have recommended for the treatment of diabetes mellitus since antiquity **[7]**.

Despite of advancements in the field of medicine, diabetes is still like a challenge causing mortality and morbidity in the world. Today, herbal products are in demand for the treatment of diabetes especially type two diabetes. There are various remedies to reduce complications caused by diabetes. Several investigations have been carried out to introduce herbal formulations; due to minor side effects and low cost. *B. aegyptiaca* (*Balanitaceae*) belongs to the family, *Zygophyloceae*, with the common names like desert date, soapberry, Also the known as Hegleg or Balah El-Abeed it can be obtained from palm trees that grow in desert of the southern valley of Egypt (Halaeib and Shelateen area).. The date is dark brown in colour and the fleshy pulp of both unripe and ripe fruits is edible and eaten dried or fresh**.** Fruit and vegetables have many similarities with respect to their compositions, methods of cultivation and harvesting, storage properties and processing. Fruit in general is acidic and sugary and are important sources of both digestible and indigestible carbohydrates. The digestible carbohydrates are present largely in the form of sugars and starches while indigestible cellulose provides roughage, which is important to normal digestion **[8]**. Balanites extract reduced blood glucose level by 24% and significantly decreased liver glucose-6-phosphatase activity in diabetic rats and suggested that the hypoglycemic effect of Fenugreek and Balanitesis mediated through insulinomimetic effect as well as inhibition of intestinal *α*-amylase activity**[9]**. The results from **[10]** showed that *B. aegyptiaca* kernel cake has an antihyperglycaemic and antihyperlipidemic effect and consequently may alleviate liver and renal damage associated with Alloxan -induced diabetes mellitus in rats. Recently, **[11]** resulted that, a realistic, compound-based rational for the anti-diabetic principle of B. aegyptiaca fruits, identified as trigonelline. Additionally, they present an additional evidence for the efficacy and complementary of NMR fingerprinting vs the standard MS-method.

This study investigates the effect of 30 days of fruits *B. aegyptiaca* ethanolic extract as the antihyperglycemic. To evaluate the antidiabetic effect of *B. aegyptiaca* ethanolic extract on pancreatic islet regeneration in type 2 diabetes (insulin independent)for induced diabetic rats by Streptotocin (STZ).Furthermore identified effect of bioactive compounds from *aegyptiaca* ethanolic on morphology of pancreatic tissues.

MATERIALS AND METHODS

Chemicals:

Streptotosin(STZ)was purchased from sigma chemical company, all chemical were analytical grade and purchased from sigma.

Plant materials

Mesocarp of *B. aegyptiaca* Del, was obtained from the Western desert of Egypt from (Mersa Alam) between April and August- Cairo, Egypt.

Preparation of extracts:

Fruit flesh was extracted with absolute ethanol, in a soxhlet apparatus for 10 hours according to **[12]** procedure.

Animals:

Male Wistar albino rats weighing 180g, purchased from National Research Centre, Cairo, Egypt. The rats were kept in a controlled environment and allowed free access to standard chow diet and water. The rats were divided into 5 groups, each group 5 rats:

Group 1: Normal rats received 0.9% saline 2(ml) for 30 days (normal group).by inter peritoneum (IP).
Group 2: Diabetic rats injected with STZ (50 mg/kgB.W) (diabetic group) by IP.
Group 3: injected with STZ (50 mg/kg) by IP and daily oral dose of (1.5 g/kg B.W) from Balanites ethanolic extracts, respectively for 30 days.
Group 4: injected with STZ (50 mg/kg) by IP and daily oral dose of (3g/kg B.W) from Balanites ethanolic extracts, respectively for 30 days.
Group5: injected with STZ (50 mg/kg) by IP and daily oral dose of (10mg/kg B.W) from dimacron, respectively for 30 days.

Blood samples were collected from the orbital plexus using heparinized capillary tubes after 30 days of treatment. Part of blood was collected on EDTA for hematological studies and the other part were collected and centrifuged for 10 min. (5000 rpm). The supernatant (serum) was immediately separated for biochemical analysis.

Induction of diabetes:

Diabetic rats were achieved by injection of freshly prepared STZ (50 mg/kg) in 0.1Mcitrate buffer at pH 4.5. **[13]**.

Chemical composition of *Balanitesea egyptica* mesocarp:

The mesocarp of Balanitesea egyptica was quantified for their moisture, crud protein, crud fat and crud fiber **[12]**. Mannose, Fructose and Sucrose were determined according to**[14]**. Total carbohydrate content was obtained by difference in order to achieve 100 g/100 g of total composition **[15]**.

Body weight of rats:

Body weight of rats was recorded at the beginning and the end of the experiment.

Serum biochemical measurements

Serum glucose was estimated by the enzymatic colorimetric method described by **[16]** after24hr.from the moment of injected with STZ and the end of experiment. Triglycerides (TG), cholesterol (TC) HDL-cholesterol and LDL- cholesterol were determined by the end of experiment as described by **[17-21]**.

Liver glycogen:

Liver was dissected out rapidly, washed with cold saline, blotted dry with filter paper and weighed. Portions of liver (100 mg) were immediately digested in 2ml of concentrated 30% KOH solution and were used for determination of glycogen content. The glycogen content of the liver was determined by an throne method as described by **[22]**.

Histopathological examinations

Pancreata were excised, and then fixed in 10% neutral buffered formalin. Tissues were then processed for paraffin embedding, subsequent serial sectioning, and stained with hematoxylin /eosin (H&E) to allow the assessment of pancreatic islet morphology according to **[23]**.

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Statistical analysis

Statistical analysis of data was achieved by busing analysis of variance and least significant difference (L.S.D) according to the method outlined by **[24]**.

RESULTSAND DISCUSSION

Proximate chemical composition has been illustrated in Table (1).The mesocarp of fruits of B. aegyptiaca was analyzed for its chemical constituents via estimation of different fractions of carbohydrates present in the fruit mesocarp. when compared to that of **[25]**. The high content of fructose and mannose in the mesocarp as observed

Table (1): Proximate Composition of Mesocarp of *B. aegyptiaca*

Parameters	Proximate chemical composition %
Moisture content	27.43
Ash	5.0
Crude fiber	2.17
Crude protein	3.35
Carbohydrate	63.21
Crude fats	3.18
Mannose	19.107
Fructose	15.702
Sucrose	6.98

In this study may be advantageous for diabetes patients who may utilize fructose as source of energy since no insulin is required for its transportation **[26]**.While the low crude fibre makes the fruit suitable to be included in low rough diet therapy **[27]** and this also improve the digestibility of protein and thus increasing the utilization of the associated amino acids **[28]**.The total crude protein content revealed possibility of the sample to be a better substitute which may incorporate in a low protein diet required to patient for **[27]**.

Table (2): Effect of *Balanitea egyptiaca* mesocarb ethanolic extract administration on serum glucose (mg/dl) for normal and -in sterptoztoicn induced diabetes in rat

Treatment	Serum glucose (mg/dl)after24hr from STZ injected*	Serum glucose (mg/dl)after30days from STZ injected*
Normal group	120±3.50 ^{b}	125±2.32 ^{d}
Diabetic group	375.4±3.60 ^{a}	420±5.33 ^{a}
STZ+1.5gBalanite extract /kg B.W	379.8±4.36 ^{a}	310±3.21 ^{b}
STZ+3gBalanite extract /kgB.W	380.4 ±4.85 ^{a}	220±5.58 ^{c}
Diamicron (10mg/kgB.W)	373.8 ±4.24 ^{a}	208±5.3 ^{c}
LSD(0.05)	12.21	14.83

*Each value represents the mean of five replicates ± S.E.The various superscript letters indicate statistically significant differences in the Duncan test, with $p< 0.05$.

Serum glucose level in SZT-diabetic rats, STZ+1.5gBalanite extract /kg B.W, STZ+3gBalanite extract /kg B.W and dia micron rats compared to normal rats is shown in Table (2) Oral administration of both dose of Balanites ethanolic extract for 30 days to STZ-diabetic rats shaved significant decrease in blood serum glucose level by 18 and 42% respectively. The diamicron (10mg/kg B.W) drug too reduced serum glucose via its pronounced hypoglycemic effect. Consistent with **[9]** daily oral administration of Balanits extract to alloxan – diabetic rats for 4 weeks was reported to reduce glucose level by 36%. Aldose reductase is a key enzyme in the

polyol pathway, which catalyzes the conversion of glucose into sorbitol in cases of hyper-glycemia **[3]**. So, aldosereductase inhibitors could be used for the treatment and prevention of such diabetic.

Table (3): Effect of *Balanitea egyptiaca* mesocarp ethanolic extract administration on body weight (g) for normal and -in sterptoztoicn induced diabetes in rat

Treatment	Initial Weight (g)*	Final weight(g)*
Normal group	183 ±1.14 [a]	218.4±1.21 [a]
Diabetic group	183.6 ±0.98 [a]	155±2.02 [e]
STZ+1.5gBalanite extract /kg B.W	183.6 ±0.81 [a]	176±1.38 [d]
STZ+3gBalanite extract /kg B.W	183.4 ±0.60 [a]	187±1.00 [c]
Diamicron(10mg/kgB.W)	181.6 ±0.68 [a]	196.2±1.77 [b]
LSD(0.05)	2.55	4.49

*****Each value represents the mean of five replicates ± S.E. The various superscript letters indicate statistically significant differences in the Duncan test, with p< 0.05.

complications**[29]**.The aldose reductase inhibitory activity of *Balanites* fruits is due to the steroidal saponins present .HPLC chromatographic profiles of the crude butanol fraction and its 4sub-fractions showed that the most highly bioactive fraction D contained the highest amount of steroidal saponins (75%) as compared to the 21 present in the original butanol fraction. The isolated furostanolsaponins proved to be highly active in an *in vitro*assay **[30]**.

Results of the effect of different ethanolic extracts 1.5 g/kg/dayand3g/kg/day, diamicron 10 mg/kg /day on body weight of STZ induced diabetic rats at the beginning and after 30 days are presented in Table (3).Diabetic rats gained less body weight with significant reduction (28%) ($P < 0.05$) to the normal control rats. The ethanolic extract of 3g/kg/day improved the body weight of diabetic rats with significant increase (18%) ($P < 0.05$) compared to the diabetic control rats, while diamicron reduced ($P < 0.05$) the body weight of diabetic rats (10%) compared to diabetic control rats.

Diabetic rats reduction in body weight is a phenomena associated with hyperglycemia. Protein-energy wasting accompanying hyperglycemia has been attributed to altered glucose metabolism as indicated by**[31]**in their experiments with diabetic rats where failure of body cells to utilize glucose as energy source seems to have proteins as an

Table (4): Effect of *Balanitea egyptiaca* mesocarb ethanolic extract administration on lipid profile parameters for normal and -in sterptoztoicn induced diabetes in rat

Treatment	cholesterol	TG	LDL	HDL
Normal group	64.4±2.01 [d]	84±1.50 [d]	15.6±0.04 [c]	33±1.67 [a]
Diabetic group	87±0.71 [a]	184.6±1.60 [a]	24.2±1.56 [a]	21.2±1.46 [c]
STZ+1.5gBalanite extract /kg B.W	77.6±0.74 [b]	103.2±1.80 [b]	19.8±073 [a]	25.6±1.08 [b]
STZ+3gBalanite extract /kg B.W	72.2±1.28 [c]	96±1.76 [c]	16.4±0.51 [b]	29.6±1.03 [a]
Diamicron(10mg/kgB.W)	68.6±0.87 [c]	93.4±2.5 [c]	16.8±0.86 [c]	30±0.707 [a]
LSD(0.05)	3.61	5.51	2.96	3.65

*****Each value represents the mean of five replicates ± S.E. The various superscript letters indicate statistically significant differences in the Duncan test, with p< 0.05.

Alternative energy source leading to metabolic imbalance in protein metabolism with consequent loss of body weight or continuous excretion of glucose from the body **[32]**.

Table(4)shows that there was significant hyperlipidemia due to experimental diabetes mellitus. *B. aegyptiaca* extracts reduced serum cholesterol, triglycerides, LDL levels and increase HDL levels significantly ($p<0.05$) than diabetic control. Diabetic rats exhibited abnormalities in lipid metabolism as evidenced from the elevated levels of cholesterol, triglycerides and high levels of low density lipoprotein cholesterol and low levels of HDL .The hyperlipidemia is associated with diabetic state **[33]** . This altered serum lipid profile was reversed towards normal after treatment with the plant extracts. The possible mechanism through which the plants exert their anti- hyperlipidemic effect might include the changed activity of cholesterol biosynthesis enzymes, changed level of lipolysis, which is under the control of insulin or direct hypolipidemic effect mediated through other mechanisms **[34]**.

Table (5): The effect of administration of *B. aegyptiaca* mesocarb ethanolic extracts on liver glycogen content

Treatment	Glycogen
Normalgroup	38.783 ± 0.16^{a}
Diabetic group	19.054 ± 0.63^{e}
STZ+1.5gBalanite extract /kg B.W	22.29 ± 0.41^{d}
STZ+3gBalanite extract /kg B.W	24.1 ± 0.68^{c}
Diamicron(10mg/kgB.W)	29.75 ± 0.77^{b}
LSD(0.05)	1.69

*****Each value represents the mean of five replicates ± S.E.The various superscript letters indicate statistically significant differences in the Duncan test, with $p< 0.05$.

Herein, the liver glycogen content in ethanolic extracts of Balanitesa egyptiaca (1.5and 3g/kg B.W) was compared to the normal show in Table(5). Oral administration of Balanites extracts to stz-diabetic rats was significantly increased glycogen content in the diabetic rat liver. The results were recorded significant decrease liver glycogen content , hyperglycemia to increase hepatic glucose production, a decrease in peripheral glucose uptake, and significant decrease in the conversion of glucose to glycogen in the liver[35].

The results indicate that pancreas histopathology of rats in groups (1,2,3,4 and 5).The Fig(1) Photomicrograph of pancreas throughout control rat (Group 1) showed strong reaction in insulin-positive-cells of islets of Langerhans were stained with anti-insulin antibody (X 400). While Fig (3 to5) Photomicrograph of pancreas of rat from group 3, 4 and5 showing numerous insulin-positive-cells of islets of Langerhans were stained with anti-insulin antibody (X 400) compared with control rats group1, Photomicrograph of pancreas showing strong reaction in insulin-positive-cells of islets of Langerhans were stained with anti-insulin antibody (X 400). **[36]** Suggested that *B. aegyptiaca* treated rats, this plant might be inducing betatrophin secretion from the liver and adipose tissues where this hormone was secreted into the blood stream to signal β-cells in the pancreas to reproduce.

Effect of *Balanitesa egyptiaca* mesocarp ethanolic extracts on *β*- cells histopathology in rats.

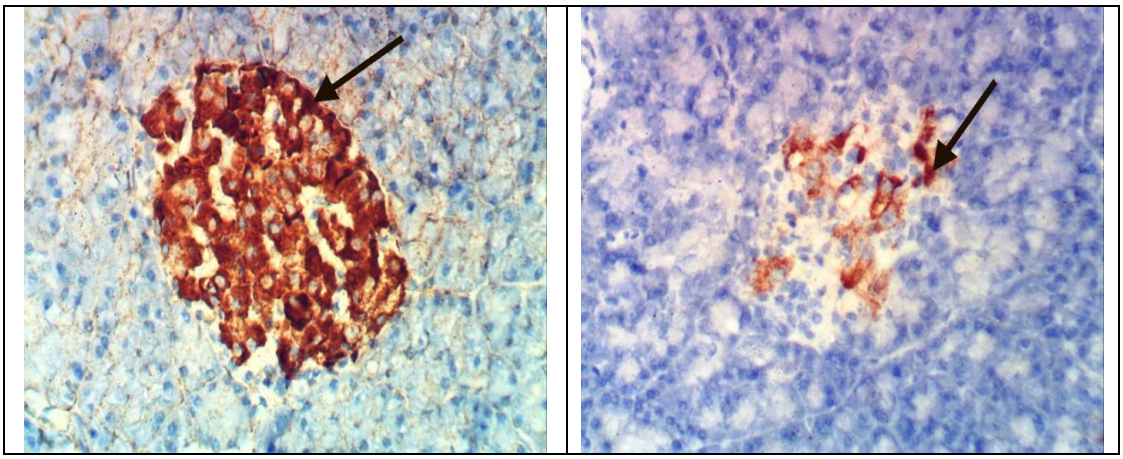

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Fig. (1): Pancreatic Photomicrograph for control rat (Group 1) (X 400).

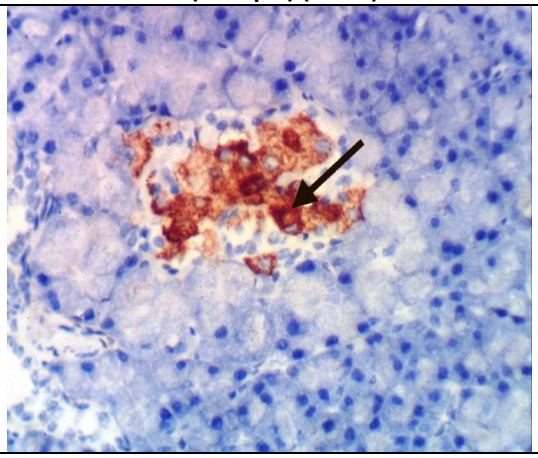

Fig. (2): Pancreatic Photomicrograph for control rat (Group2) (X 400).

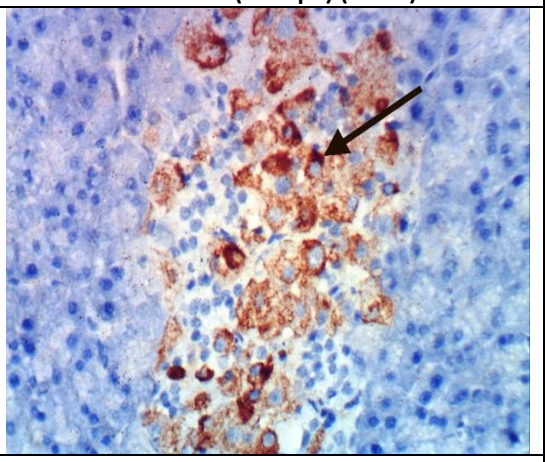

Fig. (3): Pancreatic Photomicrograph for control rat (Group3) (X 400).

Fig. (4): Pancreatic Photomicrograph for control rat (Group4) (X 400).

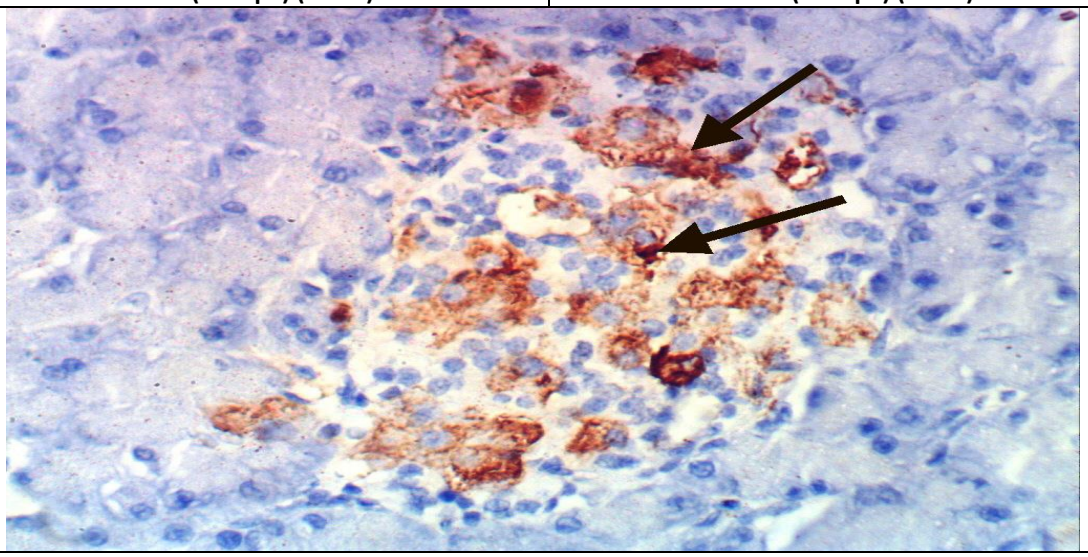

Fig. (5): Pancreatic Photomicrograph for control rat (Group5) (X 400).

CONCLUSION

The medicinal plants and their products are best substitute for the treatment of Type 2 diabetes due to their easy availability, low cost, minimum side effects and greater acceptance amongst the users. This study indicated use of ethanolic extracts of mesocarb *Balanitesa egyptiaca* exhibited anti- diabetic as well as hypolipidaemic effects on Type 2 diabetic patients.

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