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Role of *Balanites Aegyptiaca* in Attenuation of Diabetic Nephropathy

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Abstract: **Phytochemical analysis of *Balanites aegyptiaca* showed that it rich with active biological ingredients such as cumarins, flavonoids and steroidal saponins. The aim of this work is to evaluate the potential of *Balanites aegyptiaca* extract to attenuate oxidative stress and diabetic nephropathy in rats.**
Methods: Male albino rats were divided into 6 groups (each 10 rats): control rats (group1) , animals in groups (2-6) were injected *i.p* with a single dose of streptozocin (STZ), then divided into untreated diabetic rats (group 2) , diabetic treated with 0.45 mg/kg Jnuvia (group 3 as positive control) and animals in groups (4-6) diabetic treated with *Balanites aegyptiaca* at different doses 600,800 and 1000 mg/kg b.w daily by intragastric administration for 12 weeks starting from day after STZ administration.
Results: Data obtained showed that *Balanites aegyptiaca* at concentration 800 mg/kg b.w protected against oxidative damage caused by STZ as indicated by lowering of serum glucose, creatinine, tissue lipid peroxide and elevation the antioxidant enzymes activities.
Conclusion: It was concluded that, the protective effect of *Balanites aegyptiaca* may be its high contents of polyphenols and curamins. For this it can be used as complementary natural ant diabetic agent to prevent diabetic complications.

Keywords*: Diabetes mellitus- Nephropathy- *Balanites aegyptiaca.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic debilitating condition that is rapidly increasing in prevalence worldwide, as a consequence of increases in obesity, changing patterns of diet and physical activity, and ageing populations. The World Health Organization estimated that 154 million people in the world had DM at the beginning of the 21st century. DM is a metabolic disorder characterized by hyperglycemia. The hyperglycemia is caused as a consequence of a deficiency in insulin in type 1 diabetes (T1D), and is a feature of late type 2 diabetes (T2D) along with insulin resistance. T2D is significantly more prevalent than T1D. Molecular path physiological mechanisms that precede hyperglycemia, or are observed with the clinical symptoms of DM, include alterations in lipid and amino acid metabolism (1), changes in hormone levels as insulin (2) and adiponectin. The complications of DM include cardiomyopathy, vasculopathy, neuropathy, nephropathy and retinopathy, and are major causes of morbidity and mortality.

As the main goal of antidiabetic therapy is to reduce blood glucose and HbA1c levels via the administration of insulin (T1DM) or antidiabetic medication (T2DM) Furthermore, lifestyle changes, such as eating a more healthful diet, performing regular physical activity, achieving a normal body weight and smoking cessation, are recommended for

diabetic patients.Whereas the diagnosis and treatment of manifest diabetes have been thoroughly investigated, the identification of novel pathways or early biomarkers indicative of metabolic alterations or insulin resistance related to the development of T2DM is still underway. Data from the National Health and Nutrition Examination Survey showed that estimated 57.9 % of subjects with diagnosed diabetes are affected by one or more macro- or microvascular complications (3), which highlights the need for early screening markers to monitor the development of T2DM.

Balanites aegyptiaca is a plant commonly used in Antidiabetic effect, commonly used in Egyptian folk medicine as a hypoglycemic agent (4). However, there are very few studies concerning the effect of as antidiabetic agent. Antidiabetic and and Antihyperlipidemi effect of Balanites aegyptiaca in rats showed the improvement of general diabetic conditions in rats treated by the extract of *B. aegyptiaca* is possibly due to recovered endocrine pancreatic tissue at both structural and functional levels. This can lead to elevated insulin level and improved insulin sensitivity that lowers the concentration of glucose in blood. Where, insulin inhibits hepatic glucose production, stimulates both of glucose uptake and of metabolism by muscle and adipose tissues and increases liver glycogen content. addition, B. aegyptiaca containing diosgenin (5), which may be useful for ameliorating the glucose metabolic disorder, associated with diabetes and obesity. Where, diosgenin can be absorbed through the gut and plays an important role in the control of metabolic diseases such as diabetes and obesity was attributed the antihyperglycemic activity of B. aegyptiaca fruits to increase muscle basal glucose uptake significant insulin-like and partly glitazone-like activities in peripheral tissues. The aim of this study is to evaluate the effect of *Balanites aegyptiaca* on the hyperglycemia-induced in rats and if this can attenuate the development of diabetic nephropathy.

2. MATERIALS AND METHODS

Extraction of *Balanites Aegyptiaca:*

The cortex of dates were removed, dried in oven under a low degree, and grinded in a mechanical mixer then it was extracted with ethanol (10% w/v) at room temperature for overnight. The residue obtained was suspended in ethanol/water (1:9) and defatted with petroleum ether. The mixture was then extracted three times with butanol (50 ml). The butanol extracts were combined and the solvent evaporated under vaccum, a residue obtained is Balanites *Aegyptiaca* extract. (6)

Animals:

Nine week-old 200±20 g male Albino rats (n=60) were housed in cages and received the same basic diet pellet form purchased from Grain Silos and Flour Mills organization and tap water in a constant environment (room temperature (28±2) ^{o}C, room humidity (60±5%)) with a 12-h light, 12-h dark cycle. The animals were kept under observation for one week prior to the start of the experiments. All procedures will be done according to the Animal Ethics Committee At King Abdulaziz University. Ten rats were randomly selected as control group (group 1), which were treated with saline. The other 50 rats were received a single dose of Streptozotocin (STZ) (Sigma) in citrate buffer pH 4.5 at a fixed dose of 65 mg/kg. Only rats with blood glucose higher than 250 mg/dl after two days were considered as being diabetic in the fasting test. Blood glucose was measured by using single touch glucometer (purchased from Mendor Company) . Rats with blood glucose levels of less than 200 mg/dl were excluded from the study. All studies were carried out two days after STZ had been injected (7).

Experimental design:

Diabetic rats will be divided into four groups as follows:

Group II: Untreated diabetic rats.Group III: Diabetic rats treated with (0.45 mg/kg/day) januvia (hypoglycemic drug).Group IV: Diabetic rats treated orally daily with (600mg/Kg b.w) of Bal. extract.Group V: Diabetic rats treated orally daily with (800mg/Kg b.w) of Bal. extract.Group VI: Diabetic rats treated orally daily with (1000mg/Kg b.w) of Bal. extract.Bal. Extract was dissolved in distilled water and given by stomach syringe (purchased from BD Medical) .The experiment administration for 12 weeks starting from day two after STZ administration.

Blood and kidney tissue collection:

At the end of experimental period blood samples were collected under light ether anesthesia directly from the heart. The blood in plain tube was centrifuged at 3000× g for15 min and the serum was then frozen at (-80) °C until used for determination of the level urea, potassium, sodium, total protein and albumin using commercially available diagnostic kit.

After decapitation of the rats, left Kidneys were rapidly removed, washed with ice-cold saline to remove RBCs and homogenates (10% w/v) were prepared in PBS (50 mmol/l, pH 7). A part of the homogenate was used for the determination of level of reduced glutathione (GSH), catalase activity, lipid peroxidation (MDA), nitric oxide (NO), glutathione S- transferase and catalase.The right kidneys were used for histopathological examinations. Paraffin-embedded sections were made using a microtome and stained with H&E then examined under light microscope.

Preparation of kidney homogenate:

To perform the biochemical analysis on the kidney homogenate, kidneys from different groups were dissected and rinsed thoroughly with ice-cold phosphate-buffered saline to removed blood components. Later, they were blotted dry and frozen immediately in liquid nitrogen. Every kidney tissue were cut into small pieces and washed by phosphate-buffered saline. Moreover, it was ground in a homogenization buffer {0.05 M Tris-HCl pH 7.9, 25% glycerol, 0.1 mM EDTA, and 0.32 M (NH4)2SO4} containing a protease inhibitor. The solution was sonicated in ice bath to prevent overheating for 15 seconds followed by centrifugation at 14000 rpm, 4°C for 5 minutes. The supernatant was aliquoted and stored at (-80) °C

Histopathological examination:

Kidney tissue was fixed in 10% formalin, processed routinely, and embedded in paraffin. 5µm thick sections were prepared and stained with haematoxylin and eosin (H&E).

Statistical analysis:

All group values was expressed as the mean ± SD. Data were evaluated using statistical analysis program (SPSS 11). An analysis of variance test was performed initially to test for differences in the treatment. After the analyses of variance, (post hoc test) was performed to examine whether there were any significant differences between different treatment groups. or all analyses, the level of significance was set at $P < 0.05$.

3. RESULTS

In the present study we evaluated the hypoglycemic effect of Bal. extract compared with the Junavia drug (hypoglycemic drug and consequently the its protection against diabetic nephropathy in diabetic rats induced by streptozotocin. As indicated in table 1, STZ injection resulted in a nearly 3.5-fold increase of the fasting blood sugar (FBS) in the rats of group 2 as compared with control group. In addition, diabetic rats treated with Junavia reduced the glucose level significantly (P <0.001) compared with untreated group. Bal. extract feeding at different doses (600, 800 and 1000 mg/kg b.w) markedly reduced the elevated levels of FBS (P <0.001). The most effective dose was found between 800 and 1000 mg extract compared with 600mg extract

Data in table 2 also showed that the serum creatinine, was markedly higher in the diabetic untreated group when compared with the normal control group (P< 0.01). Diabetic rats treated with either Junavia or Bal. extract lower serum creatinine compared with untreated diabetic (P<0.001). It was found that, the effect of 600 mg is better than the other concentration tested. Non-significant changes were found in serum potassium, sodium and urea in all groups compared with control.

Rats injected with STZ showed a significant reduction in the levels of serum total protein and albumin compared with control group (P< 0.001). Treatment with Bal. extract showed improvement in the levels of serum Protein and albumin. The concentration used (800 mg was most effective dose used compared with 600 mg or 1000 mg.

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Table 1: Serum level of creatinine, potassium, albumin

Animal groups / Parameters	Control Group I	Diabetes group II	Diabetes+junvia group III	Diabetes+Extract (600mg)groupIV	Diabetes+Extrac (800mg)groupV	Diabetes + Extract(1000mg) groupVI
Glucose(mg/dl) Mean ±SD	100 ± 12	370.50±92.91	224.6±157.9	257.0±94.2	205.25±172.46	196.9±56.76
P value P*	——— ———	<0.001	<0.01 <0.001	<0.01 N.S	<0.05 N.S	<0.01 <0.01
Creatinine(mg/dl) Mean ±SD	1.11±.052	2.17±.009	1.19±.012	1.7±.005	1.97±.0051	1.70±.005
P value P*	——— ———	<0.001	<0.01 <0.001	<0.01 N.S	<0.05 N.S	<0.01 <0.01
Potassium Mean ±SD	2.769±.1797	2.63±0.29	2.47±0.51	2.47±0.19	2.45±0.40	2.70±0.24
P value P*	——— ———	N.S	N.S N.S	N.S N.S	N.S N.S	N.S N.S
Sodium Mean ±SD	133±13.2	135.2±6.3	138.62±2.97	137.1±5.8	134.5±3.89	138.88±2.57
P value P*	——— ———	N.S	N.S N.S	N.S N.S	N.S N.S	N.S N.S
Albumin (g/dl) Mean ±SD	3.66±0.29	2.969±0.16	3.11±0.28	2.62±0.33	3.09±0.12	2.6±0.11
P value P*	——— ———	<0.001	<0.01 <0.001	<0.01 N.S	<0.05 N.S	<0.01 <0.01
Total protein(g/dl) Mean ±SD	46.7±6.7	36.7±6.7	39.2±6.3	43.9±4.5	41.3±4.8	40.7±5.9
P value P*	——— ———	<0.001	<0.01 <0.001	<0.01 N.S	<0.05 N.S	<0.01 <0.01
Urea (mg/dl) Mean±SD	22.60±3.2	25.30±4.6	26.75±7.2	27.90±6.15	27.25±4.4	23.0±3.5
P value P*	——— ———	N.S	N.S N.S	N.S N.S	N.S N.S	N.S N.S

P value control versus all other groups

P* value diabetic treated with B. extract versus junvia treated. N.S. Non significan

The activity of antioxidant enzymes Glutathione S-transferase and catalase, as well as the concentration of reduced GSH were markedly reduced, whereas the concentration of MDA was markedly increased in the kidney homogenate of the diabetic untreated rats (group 2) compared to the control group (group1) ($P< 0.0001$), suggesting that these rats suffered from oxidative stress (Table 2). As a result of *blantica* extract feeding at different doses, these altered parameters were significantly improved in the animals ($P< 0 .01$) compared with untreated but does not return to the normal value. the most effective dose was 1000mg compared with other doses. The obtained data showed that *Balanites egyptica* extract ameliorated oxidative stress in the diabetic rats.

Table 2: The activities of antioxidant enzymes in kidney tissue (glutathione S transferase (GST) and catalase) and reduced glutathione in all studied group (Mean ± S.D)

Animal groups / Parameters	Control Group I	Diabetic group II	Diabetic+junvia group III	Diabetic+Extract (600mg)groupIV	Diabetic+Extrac (800mg)groupV	Diabetic + Extract(1000 mg) groupVI
GST Mean±SD	85.7±8.3	67± 4.8	51.5±3.4	53.3±4.6	58.12±8.01	62.9±4.2
P Value P*	——— ———	<0.01 ———	<0.001 <0.01	<0.001 <0. 01	<0.001 <0.01	<0.001 <0.01
Catalase Mean±SD	52.03±5.8	40.9±4.9	43.76±8.8	46.7±2.01	42.33±4	51±8
P Value P*	——— ———	<0.001 ———	<0.001 <0.05	<0.001 <0.01	<0.001 <0.01	<0.001 <0.001
glutathione reduced Mean±SD	13.±1.7	11.6±2.3	9.1±1.9	9.7±1.7	9.4±1.7	9.4±1.5
P Value P*	——— ———	N.S ———	<0.001 <0.001	<0.001 <0.001	<0.001 <0.001	<0.001 <0.001

P value control versus all other groups

P* value diabetic treated with B. extract versus junvia treated.

N.S. Non significan

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Lipid peroxidation marker (malondialdialhyde), nitric oxide and total proteins in kidney tissue of different groups studied (table 3). It was found that, there was a marked elevation of tissue MDA and NO in diabetic rats compared with normal control group (p<.0001). diabetic rats treated with either Junavia or balanites egypetica extract tend to normalize these parameters but don't reach to normal values. The concentration 1000mg was more effective than other concentrations used. Non significant changes in the level of kidney total proteins in all studied groups compared with control.

Table 3: The levels of malondialhyde (MDA), nitric oxide (NO) and total protein in kidney tissue

Animal groups Parameters	Control Group I	Diabetic group II	Diabetic+junvia group III	Diabetic+Ext (600mg)groupV	Diabetic+Ext (800mg)groupV	Diabetic+Extract(1000mg)groupVI
MDA (umol/g) Mean±SD	95.5±4.9	183.2±26.0	167.24±19.3	188.15±29.6	120.22±14.7	105.2±19.6
P Value P*	---------- ----------	<0.05 N.S	<0.01 N.S	<0.01 <0.001	<0.001 <0.001	<0.01 <0.001
NO(ug/g) Mean ±SD	32.4±5.60	152.65±14.3	102.7±24.805	118.745±24.713	176.03±15	94.9±27
P Value P*	---------- ----------	<0.05 ----------	<0.01 N.S	<0.01 <0.001	<0.001 <0.001	–<0.01 <0.001
Total protein(g/g) Mean ±SD	0.58±0.27	0.53±0.24	0.52±0.13	0.50±0.21	0.57±0.20	0.52±0.27
P Value P*	---------- ----------	N.S N.S	N.S N.S	N.S N.S	N.S N.S	N.S N.S

P value control versus all other groups

P* value diabetic treated with B. extract versus junavia treated.

N.S. Non significant

Histopathological examination of kidney tissue

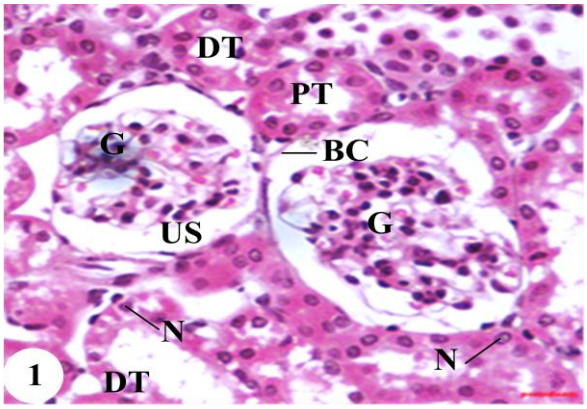

Fig.1

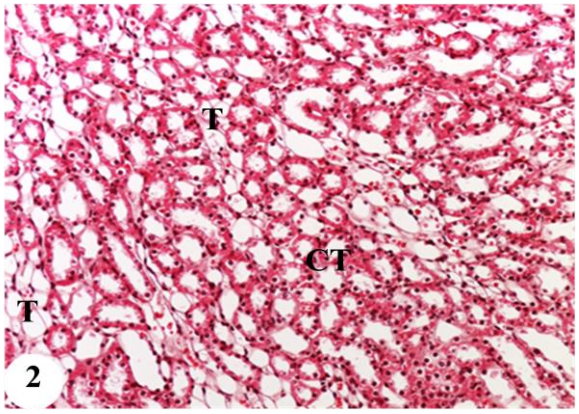

Fig.2

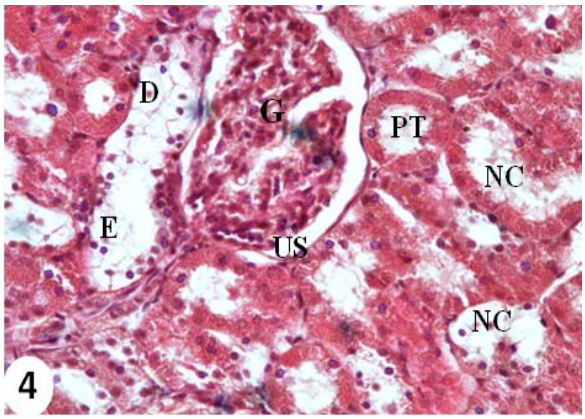

Fig.3

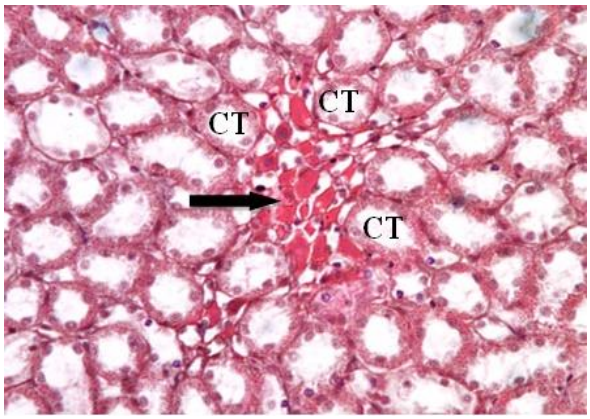

Fig.4

Histopathological examinations revealed that, Figure1 showed a section of control rat Kidney (H and E X100). The slide showing normal glomeruli (G) with an Bowman' capsule (BC), Proximal convoluted tubule (PT), Distal convoluted tubule(DT), Urinary space (US) and thickly squamous epithelium and normal nuclear. Figure 2 represent normal Collecting tubule (CT) and Thin limb of Henele Loop (T). Figure 3 showed a section of Diabetic rat Kidney (H and E X100). The slide refere a damaged cell (D), empty cytoplasm (E), narrow urinary space (US) and surrounded by extensive tubular necrosis (NC). Figure 4 infilterating white cells (arrow) between Collecting tubule (CT).Figure 5-C showing extensive tubular necrosis in Proximal convoluted tubule (PT) and distal convoluted tubule and Leaked blood cell (arrows) between tubules.

4. DISCUSSION

Diabetic nephropathy (DN) is the most common cause contributing to end-stage renal disease (8). The chronic hyperglycemia destroys function and structure of the kidney, leading to albuminuria which in turn further damages the renal tubular structure (9). In diabetes, the kidney is a direct target to the enhanced glucose levels. Advanced glycation end products (AGE) are heterogeneous products formed by the non-enzymatic reactions between reducing sugars and free amino groups of proteins, lipids and nucleic acids (10).

Administration of STZ to the rats results in a marked increase in serum glucose and creatinine and non significant changes in serum urea potassium and sodium compared with control group. The obtained data were in line with other data obtained by (11). *Balanites aegyptiaca* administration significantly attenuated the higher kidney function parameters and increased the decreased glucose and creatinine in a dose dependant manner with an optimal concentration at 600 mg/kg/day.

The inflammatory response to STZ administration is characterized by an influx of neutrophils and monocytes, up regulation of proinflammatory cytokine and chemokine expression, and increases in urinary cytokine and chemokine excretion. NO has been shown to contribute to either ischemic or toxic acute renal failure. The concentration of NO in blood or in kidney homogenate was significantly increased in the STZ- treated animals compared with the control group. Treatment with *Balanites aegyptiaca* attenuated this increase in NO levels in a dose dependent manner. This may be due to the anti-inflammatory effect of *Balanites aegyptiaca*.

Increased levels of reactive oxygen species (ROS) following the STZ treatment can be explained by several observations. First, STZ reduced the activities of antioxidant enzymes such as catalase and GST. Second, Karotalou and Essigmann demonstrated that GSH, which is the major cellular ROS-removing antioxidant within cells, is depleted in STZ treated cells. Third, STZ inhibits the respiratory chain in mitochondria, which can result in enhanced superoxide formation (12-13). Based on these observations, oxidative stress is thought to be one of components participating in STZ induced oxidative stress. The radical scavenger activity of *Balanites aegyptiaca* was due to its active ingradiant content including saponin, furanocoumarin, and flavonoid namely quercetin 3-glucoside, 3-glucoside, and, 3-7-diglucoside (14-16)

The high concentration of MDA may be attributed to the depletion in the GSH and the decreased activity of antioxidant enzymes in the STZ treated group. Decreasing the activity of catalase and GST could initiate and permit the lipid peroxidation in the cisplatin treated group. The decreased activity of catalase may be due to ROS which cause inactivation of the enzyme protein or due to the loss of zinc and copper that are essential for the enzyme activity.The results of this study concluded that administration of *Balanites aegyptiaca* gives a significant protection against STZ–induced oxidative renal damage. This is supported by results of histopathological examination that revealed a significant improvement of the ultra structure of the kidney and it is dose dependent. This protection may be due to the antioxidant- and radical scavenging- activity of *Balanites aegyptiaca* or by preventing the decline of the renal antioxidant status. In our study we indicated the potential use of *Balanites aegyptiaca* in preventing STZ -induced renal toxicity in clinical practice. In the present study diabetes induced by STZ produced impairment in renal function which manifested by increased serum creatinine and decreased total protein and albumin. Feeding with *Balanites aegyptiaca* significantly reversed the alterations of renal function and confers a hypoglycemic effect which contributes at least in part to a reversal of renal dysfunction (16-20).

In conclusion, we provide evidence that ROS scavenging is an effective approach for the prevention of diabetic nephropathy. *Balanites aegyptiaca* is a paradigm food supplement with a broad spectrum of beneficial effects, based on its ability to reduce the sequelae of hyperglycaemia-induced ROS overproduction. Since *Balanites aegyptiaca* also has beneficial effects on other target tissues as kidney, and shows beneficial effects of mediators of large vessel damage, this concept appears attractive for the prevention or delay of diabetic nephropathy.

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