



Antidiabetic and Antineuropathic Potential of Ajwa Pit & Pulp (*Phoenix dactylifera*) in Alloxanized Diabetic Rats

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ABSTRACT

Introduction: Generally in the world, diabetes is the most significant cause of deaths and morbidity. Blood glucose lowering agents possess remarkable adverse effects like weight gain and hypoglycemia and thus hindering the achievement of glycemic control. Since earliest times till today, herbal preparations have been utilized for management of diabetes and its complications. **Aims & Objectives:** To investigate antihyperglycemic and nephroprotective effects of Ajwa pit and pulp in alloxanized - diabetic and neuropathic rats. **Place and duration of study:** This study was conducted at Postgraduate Medical Institute, Lahore for the duration of four weeks. **Material & Methods:** Adult male rats (weighing 120-150 g), total of 32 were purchased and divided into 4 groups. The group1 was normal control whereas group2 was diabetic control. Diabetes was induced in groups 2, 3 and 4 with 150mg/kg single alloxan monohydrate I.P Injection. Groups 3 and 4 were given Ajwa pit and pulp rations for 4 weeks. Blood and urine samples were taken on zero and 4 weeks for fasting blood glucose, serum and urine biochemical variables for renal functions. **Results:** The results revealed that Ajwa pits significantly decreased serum levels of glucose (252±60 vs. 348±67 mg/dl), serum urea (38±7.0 vs. 63±9.6 mg/dl), serum creatinine (0.39±0.1 vs. 0.58±0.1 mg/dl) and microalbuminuria marker (7.8±1.7 vs. 9.0±2.2mg/l). Moreover it improved urine creatinine (14.0±2.9 vs.10.0±2.7 mg/dl) and creatinine clearance (0.226±0.08 vs 0.117±0.04 ml/min) when compared to diabetic control rats. Effect of Ajwa pulp was far less than Ajwa pit. Ajwa pulp did not significantly decrease serum levels of glucose (290±60 vs. 348±67 mg/dl), urea (51±9.5 vs. 63±9.6 mg/dl), creatinine (0.66±0.2 vs. 0.58±0.1 mg/dl) and microalbuminuria marker (8.3±2.0 vs. 9.0±2.2mg/l). Also there was no improvement in urine creatinine (11.1±1.6 vs. 10.0±2.7 mg/dl) and creatinine clearance (0.130±0.05 vs 0.117±0.04 ml/min) levels when compared to diabetic control rats. **Conclusion:** Ajwa pit possesses strong antihyperglycemic and protective effect in renal damage by prolonged diabetes mellitus. Highest polyphenols, bioflavonoids and antioxidants are presumed to be responsible for this effect.

Key words: Hyperglycemia, Ajwa pit, polyphenols, nephropathy, diabetes.

INTRODUCTION

The current estimates by International Diabetes Federation (IDF) 2017 authenticate the alarming burden of diabetes in the humankind but with substantial variation across various regions and diverse income groups. Approximately there are 451 million diabetics worldwide in 2017 that are expected to rise to 693 million by the year 2045. In 2017, about 424.9 million people (aged 20 years -79 years) lived with diabetes. In low income countries, about 88% of people who suffered diabetes are below the age of 65.¹

In South Asia region, diabetes has quickly developed into most important health problems. Pakistan is the main contributor of this load. The National Diabetes Survey of Pakistan (NDSP 2016-2017) has reported the prevalence of diabetes as 26.3% in Pakistan. The likeliest justification for increase in diabetes is the socioeconomic adjustments such as higher urbanization and inactive lifestyle.²

Oxidative stress is believed to be the most important cause of diabetic complications. Emergence of free radicals decrease the synthesis of self-protective enzymes in the human tissues.³ Alloxan is most commonly used in animal studies to produce

oxidative stress induced diabetes mellitus and its renal complications.⁴ The preventive role of versatile antioxidants present in medicinal plants in diabetic renal complications has been well proven through many previous researches.

Phoenix dactylifera- Ajwa date palm is well-known for its protective effects against diseases produced by oxidative stress. The pit and pulp are rich in most soluble phenolics and flavonones, dietary fibers, minerals, vitamins and energy.⁵

Ajwa dates ranked highest in having antioxidant and free radical foraging properties. Quercetin, rutin and catechin are the main valuable flavonoids present in Ajwa pit and measure about 1.3mg/100g, 6.5mg/kg and 7.3mg/kg respectively. Total phenolics in Ajwa fruit are approximately 350mg/100g.⁶

Protective effect of Phoenix dactylifera against hyperglycemia has been investigated earlier⁷ and the mechanism behind was enhancement of insulin production and amelioration of insulin resistance along with inhibition of alpha amylase and glucosidase.⁸

In a previous study, Ajwa ameliorated gentamicin induced renal toxicity in rats.⁹ However the protective effect of Ajwa has not been explored in renal problems with diabetes.

Keeping in view the cohesive protective role of multifaceted antioxidants in chronic illnesses, and ameliorative effect of few of the varieties of dates in hyperglycemia, this study was planned to examine the effect of Ajwa pit and pulp on renal damage induced by intense hyperglycemia

MATERIAL AND METHODS

Preparation of Ajwa pit and pulp diet:

Two kg whole Ajwa date was obtained from date market Madina Munawara, Saudi Arabia. The date was recognized by Botany Department of Government College University, Lahore. A voucher specimen number GC.Herb.Bot.2954 was selected. The pits were separated from pulp and washed. Air dried pits were then ground and stored in air tight jars with label on them. Pulp was smashed finely. Ground pits (1.5 g) or pulp (7grams) was blended with 100 gram rat chow separately and pellets were made. The pellets were prepared fresh weekly and stored at dry place. This each day rat feed requirement was planned on the basis of 7 dates daily recommendation for an adult human as referred in Ahadith¹⁰ and supplied ad libitum.

Animals:

Thirty two healthy male rats, weighing 120-150g were bought from University of Veterinary and Animal Sciences Lahore and placed into four groups

having 8 rats in each group. All animals were kept in animal house, Post Graduate Medical Institute, Lahore. The controlled environment temperature was maintained at 23°C with an alternating 12 h light and day cycle. They were given regular pellet diet and water ad libitum. The study was approved by the Ethical Committee of Post Graduate Medical Institute, Lahore and data was collected in 2015.

Induction of experimental diabetes:

Alloxan (Sigma USA) was freshly dissolved in normal saline just before use and given as a single intraperitoneal injection (150mg/kg) to fasting rats of all groups except normal control. After about 72 hours, animals showing blood glucose fasting level >250 and < 500 were chosen as diabetics and divided into four groups for study.

Dosage and experimental study protocol:

Group 1 (normal control) rats were given normal saline by single intraperitoneal injection. Diabetes was induced in group 2, 3 and 4 as mentioned above. Group 2 was reserved as diabetic control group. Animals of group 3 and 4 were given Ajwa pit and pulp diet respectively for six weeks after confirmation of diabetes.

Measurement of serum & urine biochemical indexes:

Cardiac puncture was performed on overnight fasted animals to collect blood samples and twenty four hour urine sample was also collected at zero and four weeks. Fasting glucose, serum urea, serum & urine creatinine were evaluated through enzymatic method using commercial Crescent diagnostic kits. Microalbuminuria was assessed by log logit method (Randox diagnostics kits). Formula was applied for calculating creatinine clearance.

Statistical analysis:

Data was expressed mean±SD applying SPSS16. One way analysis of variance (ANOVA) as well as Tukey's test was employed to see the difference between all the groups and numerous comparisons respectively. $p < 0.05$ value was considered statistically significant.

RESULTS

Effect of Ajwa pit & pulp on body weight and serum indexes

Body weight of early diabetic nephropathic rats in our study went significantly low ($p < 0.001$) in contrast to rats of normal-non treatment group. Treatment with Ajwa pit significantly recovered ($p < 0.01$) the body weight loss when compared with diabetic-non treatment rats whereas group treated

with Ajwa pulp showed less much improvement in weight.

Serum glucose levels, urea & creatinine of early nephropathic diabetic rats accelerated significantly ($p < 0.001$) in comparison to normal rats. Treatment with Ajwa pit significantly ($p < 0.01$) improved hyperglycemia as well as nearly normalized serum urea & creatinine (all $p < 0.01$) but did not reach the normal levels. Ajwa pulp significantly decreased serum urea ($p < 0.05$) whereas the effects on other parameters was much less than pit (Table-1).

Indexes	Control (non treatment)	Diabetic (non treatment)	Ajwa Pulp treatment	Ajwa Pit treatment
Body weight in grams	209.5± 9.4	140.6± 17.3	149.9± 23.8	176.4± 22.0
Fasting glucose levels (mg/dl)	85.8± 6.3	347.6± 67.2	289.9± 59.3	252.6± 60.6
Serum Urea (mg/dl)	29.9± 5.2	63.4± 9.7	51.5± 9.5	37.8± 7.7
Serum Creatinine (mg/dl)	0.36± 0.1	0.58± 0.1	0.66± 0.2	0.38± 0.1

Table-1: Results for body weight, fasting blood glucose & serum urea at 4 weeks

Effects of Ajwa pit & pulp on urine indices

Pair wise evaluation of groups demonstrated that urine creatinine & creatinine clearance levels of diabetic animals deteriorated immensely in 4 weeks whereas microalbuminuria got raised significantly (all $P < 0.001$) in contrast to normal animals. Treatment with Ajwa pit nearly normalized urine creatinine and creatinine clearance ($p < 0.01$) while ameliorated microalbuminuria significantly ($p < 0.05$) when compared to diabetic rats with renal injury. The effect of Ajwa pulp was far less than the pit (Table-2).

Indexes	Control (non treatment)	Diabetic (non treatment)	Ajwa Pulp treatment	Ajwa Pit treatment
Urine Creatinine (mg/dl)	19.2±1.1	9.8±2.7	11.1±1.6	14.0±2.8
Creatinine Clearance (ml/min)	0.236± 0.06	0.117± 0.04	0.130± 0.05	0.226± 0.08
Microalbuminuria (mg/l)	5.5±1.1	9.0±2.2	8.3±2.0	7.8±1.7

Table-2: Results for urine creatinine, creatinine clearance and microalbuminuria at 4 weeks

DISCUSSION

Diabetic nephropathy is exemplified by severe weight loss, intense hyperglycemia and altered serum & urine indexes.

In present study, a marked decline in body weight was seen in severely diabetic rats. Treatment with Ajwa pit improved the growth and body weight and decreased hyperglycemia of animals in comparison to diabetic animals. A lot of previous studies have shown correlation between intense hyperglycemia and weight loss exclusively because of loss of tissue proteins.¹¹

Our results are in line with Abdelaziz who verified that Intense hyperglycemia caused significant weight loss and Hayani date pit suspension reduced hyperglycemia and thus ameliorated weight loss in diabetic rats in a six weeks study.¹² Maliha et al demonstrated the significant ($p < 0.05$) antihyperglycemic effect of aqueous extract of Ajwa seed in 8 weeks course¹³ and date pit bread at 10% and 15% also ameliorated ($p < 0.01$) severe hyperglycemia in alloxanized rats in 6 weeks time¹⁴ and this effect may be attributed to increased insulin secretion and antioxidative enzymes of the body.

Shadab Ahmad et al also displayed that orally taken date pulp suspension decreased severe hyperglycemia in alloxan induced diabetic animals. The dietary fiber along with fructose present in fruit date (low glycemic index) decreases the absorption rate of carbohydrates, lessens insulin demand and controls blood glucose levels.⁷

The major microvascular complication of diabetes is diabetic nephropathy and is the main reason for end stage kidney failure along with other major health problems. Intense hyperglycemia generates reactive oxygen species and this oxidative stress alters many intracellular metabolic pathways of the body and finally the progressive leads to declined renal functions. On the other hand substantial randomized data proves that recovered metabolic control can achieve normoglycemia and extensively decrease development and progression of diabetic nephropathy in early phase of the disease.

In our study, Ajwa pit improved the urine creatinine and creatinine clearance while decreased the serum urea, creatinine and microalbuminuria levels in alloxanized diabetic rats implying That Ajwa pit intake may reduce the renal injury by prolonged hyperglycemia. Our data is in accordance with a study in which oral intake of date seed suspension ameliorated the renal function parameters in 4 weeks time in diabetic nephropathic rats.¹² Date fruit extract has also shown its renal functions protective effect in 4 weeks course.¹⁵

Phoenix dactylifera has been used ever since many years. In recent times, numerous reports showed excellent results in glycemic as well as proteinuria control in the early stages of diabetic nephropathy treated through different preparations of *Phoenix dactylifera* or its extract.

Studies demonstrated that antioxidative agents in date fruits and seeds including polyphenols, flavonoids, anthocyanidins vitamin C ameliorates the symptoms of alloxan induced DN in rats such as decreasing hyperglycemia, improving urine indicators for kidney damage, decreasing albuminuria and restoring weight loss.

HPLC analysis of Ajwa date seed powder revealed that total phenolics and flavonoids are 1205 mg of gallic acid equivalent/100g fresh weight and 530 mg/100mg respectively.¹⁶ The flavones content of Ajwa fruit is 2.79mg/100mg. The main dominant flavonol in date seed is quercetin (1.35mg/100mg) while other flavonols in seeds and fruit are isoquercetin, apigenin, luteolin and rutin.¹⁷

Antioxidant activity (DPPH) scavenging activity of roasted Ajwa seed powder was 74% and is the highest 16 while for Ajwa fruit, it was 20.9%.¹⁸ Ajwa pits are higher in antioxidant activity as compared to Ajwa fruit.¹⁹

Keeping in view, we can say that presence of major life saving antioxidant phytoconstituents in Ajwa date are the basis for its nephroprotective effect in diabetes.

CONCLUSION

Our data have demonstrated that Ajwa pit reduces blood glucose level and intensity of early renal injury caused by diabetes. Thus taking collectively these results, Ajwa pit has therapeutic effects on hyperglycemic states and its renal complications.

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