REPORT

Antidiabetic effects of native date fruit *Aseel* (*Phoenix dactylifera L.)* in normal and hyperglycemic rats

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Abstract: Change in dietary pattern, sedentary life style and increasing stresses are contributing factors for high prevalence of diabetes mellitus. Diabetic complications often lead to cardiovascular diseases, hypertension and hyperlipidemia that are the leading cause death and disability all over the world. Apart from pharmacotherapy, use of antihyperglycemic medicinal food is a new aspect in diabetes management and prevention of its complications. *Phoenix dactylifera* (date palm) has been traditionally used for different diseases because of the presence of bioactive agents like anthocyanin, phenols, sterols, carotenoids, procyanidins and flavonoids however its potential as a medicinal food is still unclear. Native date fruit variety *Aseel* oral suspension was evaluated using 32 normoglycemic and hyperglycemic Sprague dawley rats with two doses (300 and 600mg/kg) against control, disease control and standard drug (Glibenclamide 2.5mg/kg). Outcomes of normoglycemic studies reveals insignificant glucose lowering effects however hyperglycemic studies (glucose challenge and Alloxan inducted hyperglycemia) demonstrates significant glucose lowering effect of *Aseel* date especially with 300mg/kg dose. Data obtained during this study reveal significant anti-hyperglycemic effects of *Aseel* dates in diabetes management however further preclinical and clinical studies are required to verify the same.

Keywords: diabetes mellitus, *Phoenix dactylifera (Aseel),* normoglycemic, hyperglycemic, Alloxan

INTRODUCTION

The prevalence of diabetes mellitus is continuously increasing and affecting a major portion of world population. Epidemiologic studies have estimated that around 2.8% of the global population was suffering from diabetes in the year 2000 and it may exceed to 4.4% by the end of year 2030. Diabetes can affects all age and ethnic groups (Xing *et al*., 2009) and its complications may lead to co-morbidities and even death because of major alterations in body homeostasis (Ivorra *et al*., 1989).

Diabetes is a chronic disease that occurs either because of decreased insulin production from pancreas or when the body cannot utilize insulin effectively. Insulin exhibits key role in glucose metabolism and energy generation. Defective insulin secretion is the main reason for chronic hyperglycemia that leads to alteration of normal physiology and serious damages to many of the body's organs and system like kidney, heart, eyes, kidneys, nerves and blood vessels (Susheela *et al*., 2008; World Health Organization , 2009).

Diabetes mellitus is one of the frequent metabolic diseases with vascular complications that ultimately lead to significant morbidity and mortality and it is included in 5 major causes of death globally (Semwal *et al*., 2008). Similar to other metabolic syndromes, there is no cure for diabetes and only management exists that focuses regulation of blood glucose levels through exercise, lifestyle changes and dietary modification along with anti-hyperglycemic drugs (insulin and other antidiabetic agents). These drugs can improve quantity and quality of life. However a number of adverse effects associated with pharmacotherapy compelled researchers to try more holistic approaches like use of medicinal foods with marked anti-hyperglycemic activity to manage diabetes.

Current research focuses upon finding of natural remedies for diabetes as potential alternatives with decreased side effects and greater acceptance among general population. Various plant based compounds with significant anti-diabetic activity were isolated and studied (Chandramohan *et al*., 2008), but researchers still continue their efforts to come up with natural anti-diabetic lead or drugs to the healthcare system.

Traditionally, dates not only served as food source but many health benefits in different disease conditions were also associated with it but due to its high sugar and increased caloric contents, dates are not considered as healthy food by majority of physicians and public (Vayalil, 2012). However, in these days, efforts have been

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made by researchers to explore tremendous health advantages of dates resulting in many *in vitro, in vivo,* preclinical and clinical studies for the isolation, identification, quantification and pharmacological estimation of various phytochemicals it contained. Nutritional and phytochemical composition of date fruit reveals that they are highly nutritious and may possess several health benefits (Vayalil, 2012).

Aseel dates are among one of the commercially important date varieties of Pakistan (Markhand, 1991; Markhand *et al*., 2010). It is considered to be a chief variety of Khairpur district, Sindh. *Aseel* is an excellent semi-dry date with suitable fruit size (Markhand, 1991; Khushk, 2004). The proximate composition and mineral profile of *Aseel* variety have been summarized in table 1. To the best of our knowledge, no *in vivo* or *in vitro* experimental studies have been conducted so far on *Aseel* variety therefore we evaluated its effects on normoglycemic and hyperglycemic states in Sprague dawley (SD) rats.

MATERIALS AND METHODS

Identification of date palm

Fresh *Aseel* dates were procured from Khairpur, Sindh. Identification was made by Prof. Dr. Anjum Parveen, Director, Plant Conservation Center, University of Karachi and G.H# 92189 was maintained in herbarium.

Preparation of aqueous date fruit suspension (ADFS)

Fresh *Aseel* dates were utilized for the preparation of ADFS. 50g of flesh was blended with 100ml of distilled water until a uniform suspension is formed. This suspension was then kept in refrigerator to be used within 3 days a new batch was prepared after 3 days to avoid microbial contamination. Oral suspension was selected because it carries all the ingredients of date fruit with ease of oral administration to laboratory animals.

Animal selection

The studies were conducted with healthy male SD rats having weight in range of 150 to 250g. Animals were given standard rat chow and kept in standard laboratory conditions of temperature (23±2°C), humidity (50-60%) and 12h light and dark cycle. The rats were then randomly divided into 4 groups of 8 animals each for the three sets of experiments. Normoglycemic blood glucose level, oral glucose tolerance test and alloxan induced diabetes mellitus tests. Before the commencement of each experiment, the rats were fasted from feeds for 18-24h, but were allowed water *ad libitum*.

Normoglycemic effect

Normoglycemic blood glucose estimation was performed to assess the effect of ADFS on normal glucose levels. 32 non diabetic male SD rats were divided in four groups. Group 1 was control received distilled water; Group 2 was standard given glibenclamide 3 mg/kg while groups 3 and 4 were treatment groups received ADFS in 300 and 600mg/kg respectively. All drugs were administered by oral route. After drug administration, the rats in each of the groups were analyzed for alteration in blood sugar at 0, 30 min, 60 min and 120 min time interval using Accu-Chek glucometer, Roche, Switzerland.

Oral glucose tolerance test (OGTT)

OGTT was carried out to evaluate ADFS effects on elevated serum glucose levels induced by glucose challenge. Same SD rats used for normoglycemic study were re-utilized for OGTT test after a washout period of 15 days. 32 male rats were randomly divided in four groups. OGTT for non-diabetic rats were performed according to the modified method of Vincent and Karr (1925). Group 1 (control), received distilled water, Group 2 (standard), received glibenclamide 3mg/kg while groups 3 and 4 (treatment groups) received ADFS at a dose of 300 and 600mg/kg respectively. All drugs were administered by oral route. After 30min of drug administration, rats in each group were orally administered 200mg/kg of glucose in form of solution followed by blood glucose estimation through glucometer at 0, 30min, 60min, 90 min and 120min time interval.

Alloxan induced hyperglycemic profile

Diabetes was induced in overnight fasted rats through a single intra-peritoneal injection of alloxan monohydrate (Sigma chemicals, USA). Alloxan was freshly prepared in normal saline and given to the rats in a dose of 150mg/kg body weight. The serum glucose level was checked at 72 h after the administration of alloxan (Ankur and Ali, 2012). Rats having random blood glucose level more than 250mg/kg were considered diabetic and utilized for the study. 32 diabetic rats were divided into 4 groups each comprising of 8 animals i.e. disease control (given distilled water), standard group was given glibenclamide 3mg/kg (Özbek, 2003), treated groups were given ADFS in the doses of 300mg/kg and 600mg/kg.

All drugs were administered by oral route. Blood was taken from the tail vein and glucose levels were measured through glucometer (Accu-Chek, Roche, Switzerland). Blood samples were estimated at 0, 30min, 60min, 90min and 120min time interval.

STATISTICAL ANALYSIS

Data entry and analysis were carried out using Statistical Package for Social Sciences (SPSS) version 20IBM 2014. Data were presented as mean ± standard error of the mean with 95% confidence interval. One way ANOVA followed by post-hoc tukey test was performed for the comparisons of values with control. For time based studies, generalized linear model with repeated measure technique was employed. Values of $P \leq 0.05$ were considered as significant and $P \leq 0.005$ as highly significant.

Table 1: Composition and mineral profile of date variety *Aseel* (Adopted from Jamil *et al*., 2010)

S#	Parameter	Proximate Quantity
1	Moisture (%)	7.2 ± 0.34
2	Ash (%)	2.19 ± 0.05
3	Crude protein (%)	41.25 ± 2.05
4	Crude lipid (%)	9.05 ± 0.42
5	Carbohydrate (%)	40.22 ± 2.01
6	Total oxalate(g/100ml)	0.538 ± 0.043
7	Crude fiber (%)	86.08 ± 3.95
8	Energy value(Kcal/100 g)	352.329
9	Sodium (mg/g)	3.135 ± 0.15
10	Lithium(mg/g)	6.15 ± 0.25
11	Calcium(mg/g)	0.45 ± 0.01
12	Potassium(mg/g)	8.88 ± 0.352
13	Magnesium(mg/g)	0.2704 ± 0.001
14	Zinc(mg/g)	0.05 ± 0.001
15	Copper(mg/g)	0.3112 ± 0.01
16	Chromium(mg/g)	0.05 ± 0.01
17	Manganese(mg/g)	0.02 ± 0.001
18	Nickel(mg/g)	0.0736 ± 0.001

Table 2: Effect of ADFS on normal blood glucose levels

Drug/dose (mg/kg)	Glucose levels (mg/dl)				
	0minute	30minute	60minute	90minute	120minute
Control	67.43±2.3	88.43±1.4	69.71±1.7	88.29±0.8	92.86±1.5
Glibenclamide 3	70.43±2.35	38.29±1.3**	45.00±1.4**	29.00±1.1**	30.14±1.6**
ADFS 300	70.86±3.3	72.29±1.4**	80.57±3.3**	80.57±3.2	79.00±2**
ADFS 600	79.86±2.8**	94.14±3.2	80.29±4.8**	92.29±6.5	96.14±5

Table 3: Effect of ADFS on serum glucose level after oral glucose challenge test

Drug/dose (mg/kg)	Glucose levels (mg/dl)				
	0minute	30minute	60minute	90minute	120minute
Control	76.14±2.5	149.57±5.1	71.14±3.5	60.43±1.7	56.86±2.3
Glibenclamide 3	69.57±3	58.71±1.4**	55.57±1.6**	52.14±1.1**	56.29±8.6
ADFS 300	68.71±2.7	82.14±1.1**	78.86±1.7	72.43±1.7**	67.57±2.3**
ADFS 600	79.00±5.6	91.86±5.6**	75.43±6.1	69.43±3.6**	66.43±3.5**

Table 4: Effect of ADFS on fasting serum glucose level of alloxan induced hyperglycemic rats

Drug/dose (mg/kg)	Glucose levels (mg/dl)				
	0minute	30minute	60minute	90minute	120minute
Disease Control	266.14±6.4	305.43±8.8	339.57±5.8	338.14±11.9	312.00±10.1
Glibenclamide 3	284.00±7.3	254.00±16.5**	205.29±9.3**	175.57±7.6**	152.14±11.9**
ADFS 300	281.43±8	317.71±6.8	261.00±14.3**	226.00±13.6**	203.29±12**
ADFS 600	275.57±5.30	350.14±11.2**	299.86±10.2**	276.57±10.5**	261.14±13.3**

n=8 Mean values ± S.E.M*; P≤0.05 significant and ** P≤0.005 highly significant as compare to control; ADFS: aqueous date fruit suspension

RESULTS

Normoglycemic model

Table 2 compares the blood glucose levels of control, standard and ADFS 300 and 600mg/kg groups at 0, 30, 60, 90 and 120 minutes in normoglycemic SD rats upon acute dosing. Both treatment and standard group alter blood sugar levels significantly against control group, however there was considerable difference in the effects of standard and ADFS groups. Animals of ADFS group showed significant decrease in blood sugar levels at 300mg/kg in comparison to control group at 30, 90 and 120 min however animals received 600mg/kg exhibited insignificant increase in blood glucose levels against control group during the study period. Whereas animals received standard drug glibenclamide showed highly significant decrease in blood glucose levels during the total period of study.

Hyperglycemic model

Table 3 compares the blood glucose levels of control, standard drug and ADFS groups at 0, 30, 60, 90 and 120 minutes following oral glucose challenge in SD rats. The result shows highly significant increase in blood glucose level at 30 min in control group upon glucose challenge i.e. 149.57±5.06mg/dl followed by continuous decline. Animals received 300mg/kg ADFS showed highly significant decrease in blood glucose levels at 30, 90 and 120 min, while a similar picture was depicted by animals received 600 mg/kg ADFS. However the most significant hypoglycemic effect was observed in standard group during the total period of study.

Alloxan induced diabetic model

Table 4 reveals fasting blood sugar of ADFS (300 and 600 mg/kg) and standard groups against normal and disease control groups (alloxan induced diabetic SD rats). Both the standard and test groups exhibit significant alterations in FBS levels against disease control group however the hypoglycemic magnitude of standard drug was more. There was initial insignificant increase in blood glucose level at 30 min in 300 mg/kg group against disease control however highly significant decrease in blood glucose levels were obtained at 60, 90 and 120 min in comparison with disease control group. Similarly 600 mg/kg group exhibits significant initial raise in sugar levels at 30 min followed by highly significant decrease in blood sugar levels against disease control at 60, 90 and 120 min respectively. However standard group exhibited highly significant decrease in FBS at 30, 60, 90 and 120 min against both treatment and disease control groups.

DISCUSSION

The effect of carbohydrate consumption on human health can be accessed on the basis of how quickly they increase blood sugar levels. This property is termed as glycemic index (GI) and consumption of foods with high GI can increase blood sugar levels rapidly (Jenkins *et al*., 2002). Various studies revealed that high GI diet may increase the risk of chronic diseases like obesity, diabetes type 2 and cardiovascular disease through the induction of hyperglycemia and hyperinsulinemia (Malik *et al*., 2010). On the contrary, low GI diet exhibit reduced rate of absorption of carbohydrate giving better control over serum glucose, decrease insulin demand and reduced blood lipids level that helps in prophylaxis and management of various associated disorders (Brand-Miller, 2003).

It is a common belief among physicians and general public that due to excess sugar content date fruit are not a healthy diet especially for long term use. Especially diabetic patients are normally discouraged to use date fruit as part of their regular diet. However research data reveals that the phytoconstituent of dates are actually associated with glucose modulation in the body i.e. they increase glucose level in hypoglycemia and reduce it in hyperglycemia (Rock *et al*., 2009; Michael *et al*., 2013) therefore to assess such effects in *Aseel* date, ADFS were administered in different doses to normal and hyperlipidemic rats.

Normoglycemic study results demonstrated mixed effects of ADFS on random glucose levels against standard and control group. ADFS in 300mg/kg dose produced significant initial raise in blood glucose level however maintained sugar levels were followed afterwards showing euglycemic effects. However with 600mg/kg dose, the increases in serum glucose levels were insignificant against control.

However OGTT results revealed there was an initial significant increase in blood glucose level (at 30 min) in normal control group upon glucose challenge followed by gradual decline but 300mg/kg dose group followed insignificant increase and then maintained glucose level. On the other hand similar picture was shown by 600 mg/kg dose group in which initial insignificant increase was followed by gradual but steady decline. Most potent anti-hyperglycemic effects were produced by standard drug in these studies.

Alloxan induced hyperglycemic study showed that both the standard and treatment groups showed significant alterations in fasting blood glucose levels in comparison with disease control group. Both the 300 and 600 mg/kg dose groups exhibit significant initial raise in sugar levels which was followed by significant decline against disease control group showing significant anti-hyperglycemic activity.

The carbohydrate content of date fruit is around 65% and majorly contains glucose and fructose in nearly equal proportion. Glucose in dates with high glycemic index (GI), can relief hypoglycemia by raising blood glucose levels quickly however fructose fraction with low GI may not increase blood sugar level abruptly (Brand-Miller, 2003; Salmerón *et al*., 1997). Therefore the net increase in blood sugar level is controlled and hyperglycemia is not produced after moderate consumptions of dates .On the other hand, high consumption of dates can shift the balance towards hyperglycemia because of increased glucose concentration in the body and might also be associated with increased isomerase enzyme activity that converts glucose to fructose in different carbohydrate metabolic pathways (Turner and Turner, 1975).

Animal diabetic model studies either by glucose challenge (OGTT) or insulin insufficiency (Alloxan model) reveals that moderate consumptions of dates fruit is associated with anti-hyperglycemia, however it has minimal effects on normal glucose levels. The reason might be that in hyperglycemic animals moderate intake of dates might trigger insulin release hence producing anti-hyperglycemic effects (Kavishankar *et al*., 2011).

Dates also contain dietary fiber in adequate quantity which has been found to decrease carbohydrates absorption from gastrointestinal tract. Dietary fibers also reduce blood cholesterol level through the same mechanism therefore dietary intake of high fiber content have been suggested by health care professionals in in effective management of hyperglycemia and hyperlipidemia (Roehrig 1988, Guillon and Champ, 2000). Another possible mechanism for anti-hyperglycemic effect of date fruit may be due to its potent antioxidant and anti-inflammatory constituents that decrease oxidative and inflammatory stress associated with increased cortisol release (Rock *et al*., 2000; Mohammed *et al*., 2007).

CONCLUSION

It appears that *Aseel* date variety possess anti hyperglycemic activity because of its phytochemicals, its low GI and high fiber content makes it an effective medicinal food in the management of diabetes, since normal and hyperglycemic studies on animal models reveals significant glucose lowering effects. However data collected during present study needs to be substantiated through preclinical studies on large number of animals as well as clinical studies.

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