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Effect of Birhi Variety of Date Palm Fruits, (*Phoenix dactylifera* L.) at the Tamr Stage on Serum Glucose Levels in Streptozotocin- Induced Diabetic Rats

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Abstract

This study was carried out to investigate the effect of a dietary preparation of Birhi tamr (*Phoenix dactylifera* L.) on serum glucose levels and body weight in normal, diabetic insulin-treated and diabetic insulin-untreated rats. Diabetes was partially induced by intraperitoneal injection of streptozotocin (32.25 mg/kg). Thirty six male Sprague-Dawley rats (239 ± 8.4 g) were divided into two normal, two diabetic insulin-treated and two diabetic insulin-untreated groups. Each group was fed a diet containing either 0% or 10% tamr for six weeks. Fasting serum glucose levels were determined by enzymatic-calorimetric method using a standard kit procedure. Feeding 10% tamr did not show significant differences ($P > 0.05$) in serum glucose levels in any of the normal and insulin-treated diabetic rats. Insulin-untreated diabetic rats fed 0% tamr or 10% tamr exhibited significantly ($P < 0.05$) higher serum glucose levels (496 ± 81.6, 315 ± 61.1 mg/dl respectively) compared to normal (147 ± 5.3, 156 ± 7.6 mg/dl respectively) and insulin-treated diabetic rats (227 ± 17.6, 268 ± 18.9 mg/dl respectively). Feeding 10% tamr to insulin-untreated rats induced significant ($P < 0.05$) reduction in this variable. The findings of the present study may provide support for the favorable effect of date palm fruits as tamr, on blood glucose in streptozotocin-diabetic rats. This effect cannot be simply explained on the basis of the macro-nutrient composition of tamr. However, due to the apparent effects of tamr on blood glucose in normal and insulin-treated and insulin-untreated diabetic rats, the possible presence of insulin-like substance in tamr may not be excluded. The mechanism of action of the blood glucose-lowering effect of tamr awaits further investigation.

Keywords: date palm fruit, Birhi variety (*Phoenix dactylifera* L.), streptozotocin (STZ), serum glucose, Sprague-Dawley rat

1. Introduction

Diabetes mellitus is a major health problem worldwide (ADA, 2002). It is a major cause of death in many countries, and presents a serious health concern in Jordan (Ajlouni et al., 1998; Ajlouni et al., 1999). The disease is associated with abnormal changes in protein, carbohydrate and fat metabolism (Dominique et al., 2003) and induce disturbances in lipid profiles especially, an increased susceptibility to lipid peroxidation (Drisko et al., 2003), as well as body weight defects (ADA, 2002). Diabetes accounts for a substantial burden of morbidity and mortality through micro- and macro vascular complications (Gaede et al., 2003; Heymann et al., 2006). The most common chronic complications of diabetes include retinopathy, nephropathy, neuropathy and atherosclerosis (Al-Shamsi et al., 2007). Animals receiving streptozotocin exhibit metabolic and clinical manifestations reminiscent of human diabetes (Szkudelski & Szkudelska, 2002).

Diet is the cornerstone of diabetes management. The aim of dietary therapy is to improve metabolic control and lessen the adverse effects of diabetes on the homeostasis of body weight and blood glucose (ADA, 2002). Efforts are now being exerted to produce a diet formula that best controls the disease and its complications. A more recent view to the subject is the attempts of introducing functional foods to the diabetic diets (Hill and

Peters, 2002). A food can be regarded as “functional” if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutrition, in a way that improves health and well-being or reduce the risk of disease (Roberfroid, 2000).

Dates (*Phoenix dactylifera* L.) have been an important crop in the desert regions of Middle Eastern countries and formed the basis of survival of many ancient nomads (Al-Hooti et al., 2002). Even today, dates continue to play an essential role in the diet of the local inhabitants (Ahmed et al., 1995). Dates are still a dietary staple of the people of this area, and are frequently referred to “the bread of the desert” (Al-Shahib & Marshall, 2003). Palms represent the third most important plant family with respect to human use (Johnson, 1998). The fruits are commonly consumed as fresh dates at the khalal and rutab stages, or as dry fruits at the tamr stage (El-Shaarawy, 1989; Ahmed et al., 1995). Dates are also consumed in other forms particularly date paste and syrup (El-Shaarawy, 1989), date chutney (Sawaya et al., 1989), tamaroggt (Al-Ruqaie & El-Nakhal, 1989) and jam (Mustafa et al., 1983a). They may also be used as a sweetening and/or flavoring agent in ice cream (Hamad et al., 1983), bakery products, juices (Mustafa et al., 1983b) and milk beverages (Yousif et al., 1989). Birhi variety of dates is the major variety produced by Jordanian farmers (Naber, 2005).

Dates contain high percentages of carbohydrates and appreciable level of dietary fibers, with some mineral elements and vitamins as well as a good protein level with twenty three different amino acids (Al-Shahib & Marshall, 2003). The fruit also contains several active phytochemicals such as polyphenols, sterols, tannins and carotenoids (Vayalil, 2002).

In spite of the long history of dates as nutritional staple in the Middle East, most of the published studies on it have dealt mainly with its nutritional value and its uses in food industry (Al-Shahib & Marshall, 2003). Dates are included in the list of the folk remedies as a dietary factor that protects against heart disease, with possible anti-diabetic effects, though scientific reasoning behind this use are not known (Amer, 1994). In clinical settings, diabetic patients are commonly advised to reduce or even avoid the ingestion of dates to prevent presumed wide fluctuations in blood glucose level. The apparent reason behind this advice is the high carbohydrate content of dates, mainly in the form of sugars. However, there is no scientific evidence available to validate such a dietary restriction.

Contrary to the usual belief, dates are classified as low glycemic index food items (Miller et al., 2002, 2003). The index varies depending on the variety and the stage of ripening. The glycemic indices in normal subjects were 35.5 for khalas, 49.7 for Birhi and 30.5 for ma'an date varieties (Miller et al., 2002); 47.2 for Khalas Rutab and 28.9 for Khalas Tamr/yoghurt preparation (Miller et al., 2003). For the commercially stored Khalas date variety, the index was 57.7 in both diabetic and normal subjects (Ahmed et al., 1991). The low glycemic index of dates may be attributed to their high content of fructose and dietary fiber, and perhaps to other micro-chemicals contained in dates.

To the best of our knowledge, no studies are available to indicate the effect of date palm fruits, incorporated as part of the diet, on blood glucose and body weight homeostasis in diabetic men and animals. Studies of the metabolic responses to dates and the effect of their incorporation into mixed diets in normal and diabetic man or animals are also lacking. This study was conducted to evaluate the effect of tamr of Birhi dates incorporated as part of the diet on blood glucose levels in streptozotocin-induced diabetic rats.

2. Methods

2.1 Date Collection and Preparation

A quantity of locally produced Birhi date from the tamr stage of ripening was obtained from a local farm in due season, 2005 (Al-Baraka Farms, Jordan). Dates were stored in sealable bags (Zepter-Vacsy bag, Korea) under vacuum (Zepter-Vacsy, Italy). The packages were then deep frozen (-20 °C) for subsequent analysis and diet preparation (Al-Mashhadi et al., 1993).

Date sample representing the tamr stage of ripening were taken, and were bitted before the flesh was ground up separately in a food grinder (National, MK-G30NR, Japan). The ground samples of tamr were dried in a drying oven (Memmert, Karlklob -West Germany) at 70 °C until constant weights were obtained. After drying, they were ground separately to powder using pestle and mortar and placed in sealed polythene bags in desiccators (Al-Shahib & Marshall, 2002).

2.2 Chemical Analysis of Dates

Proximate nutrient composition of dried Birhi Tamr was determined by using the official methods set by the Association of Official Analytical Chemists (AOAC, 1995). Moisture content of dates of tamr stages was obtained using drying-oven method. Nitrogen content of oven-dried samples of tamr was obtained using the

micro Kjeldahl method (AOAC, 1995). Crude fat content of oven-dried samples of tamr was determined using the Soxhlet extraction method (AOAC, 1995). Crude ash content of oven-dried samples of tamr was obtained using the dry ashing method. Crude fiber content of dried samples of tamr was obtained by the Van Soest method. Nitrogen-free extract that represents the soluble carbohydrates, was calculated by difference, subtracting the sum of crude protein, crude fat, crude fiber and crude ash content from 100%.

2.3 Preparation of Experimental Diet Mixtures

Two experimental diet mixtures were prepared that have isocaloric, isonitrogenous and isonutrient (proteins, vitamins and mineral elements) content. They contained 0% or 10% of tamr. The experimental diet mixtures were composed of specific amounts of dried egg albumin, corn starch, corn oil, fat-soluble vitamin mixture, water-soluble vitamin mixture, mineral mixture, DL-methionine, and fresh dates from tamr. They were thoroughly mixed in a stainless steel blender (Kenwood, Hampshire, England).

Table 1 shows the ingredient composition of the two experimental diet mixtures. The nutrient composition of the dried egg-albumin (Enthoven, Raalte, Holland) and the dried date preparations were considered in the formulation of diet mixtures. Fresh date preparations of tamr were incorporated into the diet mixtures. However, for the purpose of formulation of diet composition and calculation of nutrient intakes, the nutrient composition of dried dates was considered. The composition of vitamin and mineral mixture was according to Reeves (1997). The protein and caloric content of diet mixtures were 18% and 425 Kcal/100g respectively. Table 2 shows the calculated proximate nutrient composition of the diets used in the experiment. The experimental diet mixtures were freshly prepared twice a week and stored in a refrigerator at 4 °C until needed during the feeding stage. Diets were added daily in amounts depending on the daily intake of rats.

Fresh tamr was bitted and ground (National, MK-G30NR, Japan) just before preparing the diets. It was intended to incorporate fresh date into the diet mixtures to preserve their micro-components that might be affected by drying (Caro et al., 2004). In order to do so, known amounts of corn starch and fresh tamr were separately mixed and allowed to pass through a sieve of 1 mm mesh to obtain homogenous mixes. Specific amounts of these mixes were used in diet preparation. Mixes of starch and fresh dates were prepared just prior to diet preparation.

Table 1. Ingredient composition of experimental diets

Component	Diet mixtures (g/100g)	
	0% Tamr	10% Tamr
Albumin	18.63	18.39
Corn starch	65.07	55.81
Corn oil	9.0	8.92
Tamr	0.0	10
Water-soluble vitamin mix ⁽¹⁾	2.0	2.0
Fat-soluble vitamin mix ⁽²⁾	1.0	1.0
Mineral mix ⁽³⁾	4.0	4.0
DL-Methionine	0.3	0.3

Note. ⁽¹⁾ 1 kg water soluble vitamin mix composed of the following: 0.5 g thiamin hydrochloride, 0.4 g riboflavin, 45 g ascorbic acid, 4 g calcium pantothenate, 4 g nicotinic acid, 2.5 g choline, 25 mg inositol, 10 g para-aminobenzoic acid, 0.02 g biotin, 0.2 g folic acid, 0.4 g pyridoxine hydrochloric, 2.5 mg cyanocobalamin, and 0.225 g menadione (vitamin K). Mixture weight was continued to 1kg by dextrose (Adapted from Reeves, 1997). ⁽²⁾ Fat soluble vitamin mix composed of the following: 0.25 g retinyl acetate, 1 ml of solution made by dissolving 0.125 g vitamin D2 in 10 ml oil, 2 g vitamin E (DL- α -tocopherol acetate) in 500 ml corn oil (or 10 ml fat soluble vitamin mix provide 400IU vitamin A, 1000IU vitamin D2 and 40IU vitamin E) (Adapted from Reeves, 1997). ⁽³⁾ Mineral mix composed of the following: 0.21 g AlK (SO₄)₂·H₂O, 350 g CaCO₃, 250 g KH₂PO₄, 0.26 g COCl₂·6H₂O, 0.5 g CuSO₄·5H₂O, 9.424 g FeSO₄·7H₂O, 49.94 g MgSO₄, MnSO₄·4H₂O, 0.25 g KI, 135.48 g K₂HPO₄·3H₂O, 127.58 g NaCl, 0.0635 g NaF, 0.26 g Na₂B₄O₇·10H₂O, 1.65 g ZnSO₄·7H₂O, 0.0815 g H₃BO₃, 0.0174 g LiCl, 1.45 g Na₂O₃Si, 0.28 g CrK(SO₄)₂·12H₂O, 0.0089 g VCl₃, 5.0659 mg SeO₂, 5.04 mg MoO₃. Mixture weight was continued to 1kg by dextrose (Adapted from Reeves, 1997).

Table 2. Calculated nutrient composition of experimental diet mixtures (%)

Component	Diet Mixtures (%)	
	0% Tamr	10% Tamr
Protein ⁽¹⁾	18	18
Nitrogen free extract ⁽²⁾	65.7	65.7
Fat ⁽³⁾	9	9
Water-soluble vitamin mix	2	2
Fat-soluble vitamin mix	1	1
Mineral Mix	4	4
DL-Methionine	0.3	0.3

Note. ⁽¹⁾ Dried egg albumin, ⁽²⁾ Corn starch, ⁽³⁾ Corn oil.

2.4 Animal Experimentation

Adult male rats weighing about 200 g were individually housed in plastic cages with stainless steel wire-mesh bottoms (B. Holden and Crew 2001, North Kent Plastic Cages Ltd, England). They were fed for two weeks prior to start of the experiment for acclimatization. Environmental conditions were under control with a temperature of 24 ± 2 °C and 12 hour light dark cycle. Diets were given in glass jars and water was provided in glass bottles with rubber stoppers.

At the beginning of the experimental feeding stage, thirty six male rats (12 week old) were distributed randomly according to their weights 239 ± 8.4 g into 6 groups. Twelve animals were divided into two groups (6 rats/each group) and served as the normal groups. Diabetes was partially induced in rats by a single intraperitoneal streptozotocin (2-deoxy-2- {[methylnitrosamino] carbonyl}-amino)-D- glucopyranose) (Sigma Chemicals Co., Mo, USA) injection (32.25 mg/ml freshly dissolved in 0.05 M citrate buffer, pH 4.5 at a dose of 32.25 mg/kg) (Kanarek & Ho, 1984; Yamada et al., 2002). After 24 hours, diabetes was checked by testing glucosuria of the rats using glucose urine strips (Gikotest, Rocho Germany) and by observing polydipsia and polyuria. Rats which showed positive glucose urine results were 24 rats. The rest of the rats which showed negative glucose urine results were excluded from the experiment. Diabetic rats (24 rats) were divided into four groups (6 rats/each group). Two of the diabetic groups were treated subcutaneously with Insulatard[®] HM at a dose of 10 U/kg/day and served as the insulin-treated diabetic groups; the other two diabetic rat groups were left untreated and served the insulin-untreated diabetic groups. Each diet mixture, described earlier, was given to one normal and one insulin treated and one insulin untreated diabetic groups for a period of six weeks. Figure (1) shows the experimental arrangement of normal and diabetic rats in the study.

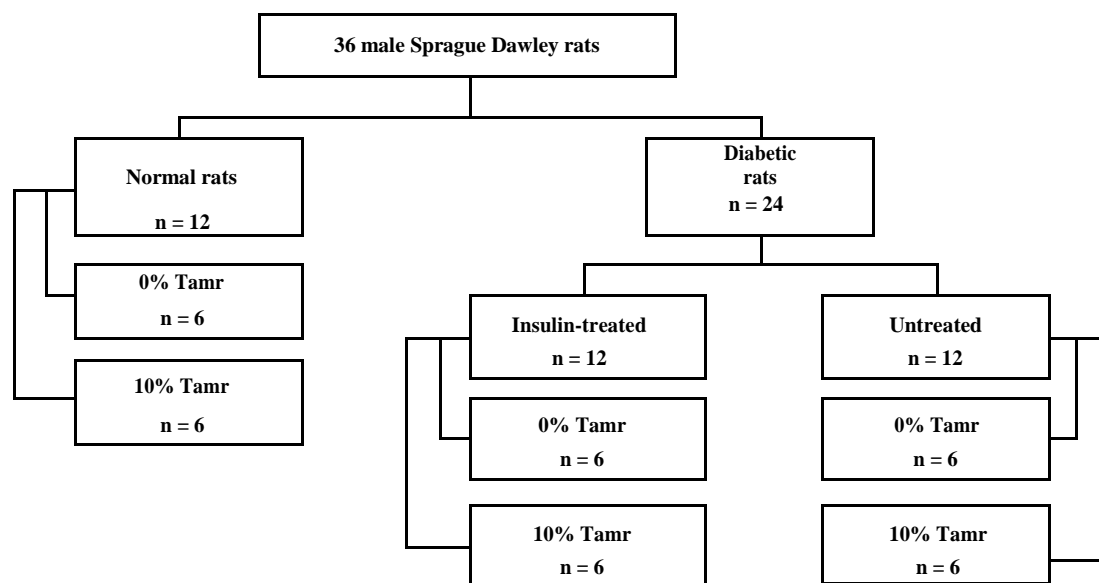


Figure 1. Experimental arrangement of normal and diabetic rats

At the end of six weeks, rats were starved for 8 hours, anesthetized with chloroform, weighed then blood was drawn from right ventricle of the heart using a medicinal syringe and transferred to plain tubes, centrifuged at 3200 rpm for 15 minutes (Clement, ES 150 centrifuge, Australia) to obtain serum. Samples of the serum were stored frozen at -20°C until analysis was performed.

2.5 Biochemical Analysis of Serum Glucose

Serum glucose concentration was analyzed in Al-Takhasosi hospital laboratory section Glucose was measured by Cayman's Glucose Colorimetric Assay Kit.

2.6 Statistical Analysis

The results of each studied variable were subjected to the analysis of variance (ANOVA) and least significant difference test using the statistical analysis (SAS package version 9) to determine if differences in means were significant at ($P < 0.05$). The results were presented as means \pm standard error of the mean (SEM).

3. Results

3.1 Proximate Nutrient Composition of Tamr

Proximate nutrient composition of tamr from Birhi dates is presented in table 3. Moisture content was 17.2 %. The proximate nutrient composition of the date tamr stage of ripening on dry matter basis were 2.4% crude protein, 0.8% crude fat, 2.0% crude fiber 2.2% ash and 92.6% nitrogen free extract. Calculated energy content of tamr was 387.2 total energy (Kcal/100 gm).

Table 3. Proximate nutrient composition of Birhi tamr⁽¹⁻³⁾

Composition	Tamr
Moisture	17.2 \pm 0.09 %
Crude protein	2.4 \pm 0.01 %
Crude fat	0.8 \pm 0.04 %
Crude fiber	2.0 \pm 0.07 %
Crude ash	2.2 \pm 0.03 %
Nitrogen free extract	92.6 \pm 0.02 %
Total Energy Kcal/100gm	387.2

Note. ⁽¹⁾ Mean values of triplicates with less than 5% coefficient of variation (dry matter basis \pm SEM). ⁽²⁾

Nitrogen free extract was calculated by subtraction of ash, crude protein, crude fat and crude fiber from 100%.

⁽³⁾ Total energy was calculated as (protein \times 4 Kcal/g + fat \times 9 Kcal/g + NFE \times 4 Kcal/g).

3.2 Blood Glucose Levels

Table 4 shows serum glucose levels of normal and diabetic rats fed 0% or 10 % tamr for six weeks. Feeding 10% tamr did not show significant differences ($P > 0.05$) in serum glucose levels in any of the normal and insulin-treated diabetic rat groups. However, insulin-untreated diabetic rat groups fed either 0% or 10% tamr diets exhibited significantly ($P < 0.05$) higher serum glucose levels compared to normal and insulin-treated diabetic rats. Feeding 10% tamr to insulin-untreated rat groups induced significant ($P < 0.05$) reduction in this variable.

Table 4. Serum glucose levels of normal and diabetic rats fed diets containing 0% or 10% Birhi Tamr for six weeks⁽¹⁻²⁾

Experimental group*		Serum glucose level mg/dl
Normal	0% Tamr (Control)	147.5 ± 5.3 ^a
	10% Tamr (Control)	156.3 ± 7.6 ^a
Diabetic	Insulin-treated (0% Tamr)	227 ± 17.6 ^b
	Insulin-treated (10% Tamr)	268.3 ± 18.9 ^b
	Insulin-Untreated (0% Tamr)	496 ± 81.6 ^c
	Insulin-Untreated (10% tamr)	315 ± 61.1 ^d

Note. ⁽¹⁾ Values are given in means ± SEM. ⁽²⁾ Values in a column with different superscripts differ significantly ($P < 0.05$).

4. Discussion

An in -depth search in the literature revealed lack of controlled studies that investigate the effect of date palm fruits on the serum glucose levels and body weight in streptozotocin-induced diabetic rats. Studies that were carried out investigate the effect of tamr on blood glucose are very limited. To our knowledge, this is the only research done to investigate the hypoglycemic effect of tamr as a nutritional entity. Such limitations make it difficult to discuss the results from a nutritional point of view. However, it may be beneficial to open new avenues of thinking for future research and studies in this field. Studies which focus on the effect of certain nutrients or chemicals that are naturally found in dates on body functions during a particular anatomic or physiologic state may be considered. Both the macro- and micro components of dates are considered. Examples of such components are vitamins, mineral elements, sugars, dietary fiber and polyphenols. It is important to emphasize that the obtained results are related to the conditions followed by the present study and any change in these conditions, especially the date material together with the animal breed and age may affect the results.

Dates "*Phoenix dactylifera*" are of high content driven from the appreciable amounts of sugars including glucose and fructose found in the flesh. Additionally, dates have considerable contents of minerals, vitamins, dietary fibers and antioxidants (Hong et al., 2006). A number of studies have been conducted to investigate the effects of date consumption on human health, which have illustrated valuable results of the consumption of this fruit in maintaining well-being and in treating certain disorders (Puri et al., 2000; Miller et al., 2002; Ishrud & Kennedy, 2005; Al-Qarawi et al., 2005).

With respect to tamr used in the present study, it was of high quality and met all the physical and chemical criteria indicated by Jordanian standard specifications (JISM, 1988) and Codex Alimentarius (1985). The maximum allowance for the defects including: blemishes, damages, and unripe dates have been determined specifically in Codex Alimentarius and Jordanian standards. There was a slight variation in the proximate nutrient composition of the date used in this study in comparison with previous studies. It may be due to the variation of date variety itself, the preservation methods, the geographical and climate differences of areas where date palm tree is grown and finally the used chemical analytical procedures (Barreveld, 1993; Al-Shahib & Marshall, 2003).

There are no reported observations about effects of date palm fruits on blood glucose levels. Streptozotocin injection play a major role in increasing blood glucose levels (Zhong, 2001; T. SzKudelski & K. SzKudelska, 2002; Namkoong et al., 2005). Streptozotocin is known to selectively destroy the pancreatic beta cells that produce insulin leading to hyperglycemia (Bonner- weir et al., 1981; Yang & Wright, 2002). Streptozotocin impairs glucose homeostasis in the liver (Hidaka et al., 2002). In the present study normal rats exhibited blood glucose levels ranged from 150-180 mg/dl which goes in line with the values reported by previous studies

(Rafferty & MacLachlan, 1941).

In this study, blood glucose concentration tended to be lower in diabetic insulin-untreated group fed 10% tamr than the group fed 0% tamr diet. This result may be attributed to the fact that dates have low glycemic index (Ahmed et al., 1991, 1998; Famuyiwa et al., 1992; Miller et al., 2002, 2003) despite their high content of sugar and carbohydrates. Glycemic index of Birhi date is 49.7 at tamr stage. The low glycemic index of dates can be attributed to the high fructose content. Table 4 shows serum glucose levels of normal and diabetic rats fed 10 % tamr for six weeks. It is shown from the table that the diabetic insulin-untreated group fed 0% tamr diet has the highest blood glucose levels.

5. Conclusion

Diabetic insulin-treated rats exhibited similar serum glucose levels compared to normal rats. It was noted that dietary incorporation 10% of tamr resulted in a decrease in blood glucose levels in diabetic insulin-untreated rats. Hence, an insulin-like substance in tamr, that improves the metabolic status of diabetic rats, is postulated.

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