EFFECT OF GRADED LEVELS OF DATES DIETARY FIBER ON WEIGHT GAIN, SERUM GLUCOSE AND INSULIN LEVELS OF NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

***Objective**: The aim of the study was to evaluate the effect of graded levels of dates dietary fiber on diabetes mellitus induced by streptozotocin (STZ) in male Sprague-Dawley (SD) rats.*

***Methodology:** Rats were divided into eight groups, among which four groups (Groups 1-4) were normal and the other four groups were diabetic (Groups 5-8). Diabetes was induced in rats by an intraperitoneal injection of STZ (50 mg/kg body weight). Different concentrations (5%, 10% and 20%) of dates fibers were mixed with standard diet and administered to rats except group 1 and group 5. Changes in body weight, food intake, water intake, serum glucose and insulin levels were observed in normal and diabetic rats on 0, 10th and 21st day. Oral glucose tolerance test (OGTT) was also carried out on day 21.*

***Results:** Body weight of diabetic control group increased but not significantly. A significant difference in the average food intake was observed between non-diabetic and diabetic groups. Though the rats in the diabetic groups consumed more amount of water, there was no significant difference when compared with non-diabetic group. Significant difference in serum blood glucose level was observed in diabetic control group (group 5) when compared to other diabetic groups. Non-significant difference in the levels of serum insulin was observed in diabetic control group compared to other diabetic groups. Serum glucose levels after OGTT were increased significantly in diabetic groups compared to non-diabetic groups.*

***Conclusion:** Addition of dietary dates fibers to standard diet and administered to diabetic rats had a potential role in reducing their food intake, reducing or controlling weight, as well as lowering blood glucose.*

***Key words:** Streptozotocin, diabetes mellitus, Sprague-Dawley rats, dietary dates fibers, serum glucose, insulin*

INTRODUCTION

Diabetes mellitus (DM) is a major public health problem worldwide, and is a well known risk factor for coronary artery disease. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.(1) It is estimated that by the year 2025 there will be about 300 million people affected with DM in the world with the highest prevalence in the developing countries.(2) Profound changes in the life style of the people of Arabian Peninsula during the last 30 years have been associated with the emergence of diabetes. A recent community-based national epidemiological health survey in KSA has found that the overall prevalence of DM is 23.7% which is alarming for health care providers.(3)

The association between diabetes and fiber depletion has been documented.(4) Recent studies have provided preliminary evidence for reduced risk of diabetes(5) and a beneficial effect on health maintenance and disease prevention(6) with increased intake of dietary fiber. As a component of medical nutrition therapy, an inverse relationship between dietary fiber intake and incidence of diabetes, obesity, heart disease, cancers and gastrointestinal disorders have been reported.(7) There are data suggesting that consuming a high-fiber diet (~50 g fiber/day) reduces glycemia in subjects with type 1 diabetes and glycemia, hyperinsulinemia, and lipidemia in subjects with type 2 diabetes.(5) Moreover, increased fiber intake appears to be desirable for people with diabetes, and a first priority might be to encourage them to achieve the fiber intake goals set for the general population of 14 g/1,000 kcal.(8)

Dates are the fruit of the date palm tree (*Phoenix dactyliferous*). It is a staple food of Arabia peninsulas. The Kingdom of Saudi Arabia (KSA) is the foremost in terms of per capita consumption.(9) Large quantities of dates of different varieties are harvested in different regions of Saudi Arabia.(10) Dates are used in breaking fast during the Holy Month of Ramadan [It is mentioned in the Holy Qur'an (Sorat Maryam 19:25-26)].(11) Dates contain a high percentage of carbohydrate and classified as low glycemic index food item.(12.13) The dietary fiber (usually insoluble) content of variety of dates has been shown to be as high as 6.4-11.5%.(14,15)

Several researches have reported the effect of different sources of dietary fibers on human health, especially on diabetes mellitus. However there are no available studies on beneficial effects of date's dietary fibers in Saudi Arabia. Considering dates

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historical, socioeconomic and nutritional values, it was of interest to study the effect of dates dietary fiber supplemented diets on different blood parameters using a rats model. Nyman et al showed good correlation between man and rat in the degradation of dietary fiber.[16] The present study investigated the effect of different levels (5%; 10% &20%) of dates dietary fiber on weight gain, serum glucose and insulin levels in normal non-diabetic and STZ-induced diabetic rats.

METHODS

Animals

A total of 74 male Sprague-Dawley (SD) adult rats weighing (250-280 g), were obtained from Animal facility, Comparative Medicine, Research Center of King Faisal Specialist Hospital, Riyadh, KSA. The rats were divided randomly into eight groups, of 8-10 rats /group. Four groups were treated with Streptozotocin (STZ) to induce diabetes. The remaining four groups were considered as non-diabetic groups.

Induction of Diabetes: Streptozotocin (STZ) has long been used as a drug of choice to induce diabetics type II in various animal models. This well-established model is characterized by insulin deficiency associated with insulin resistance.[17,18] Streptozotocin (from Sigma- Aldrich Co. Ltd. Poole, England) was diluted in 1.0 ml citrate buffer (pH. 4.4) immediately before it was injected intraperitoneally to the rats in groups 5, 6, 7 & 8. The nondiabetic rats in groups 1, 2, 3, & 4 were injected with 1.0 ml citrate buffer alone / kg body weight.

Experimental Diets: The experimental diets were prepared according to AIN-93G purified rodent diet, as modified by Reeves et al (1993).[19] The standard diet (Rodent Laboratory Chow, basic nutrients need) was obtained from (Grain Silos & Mills Organization, Laboratory Animal, Riyadh) and it was used for the control groups (1 & 5). The dates fiber were added in different concentrations (5%, 10%, 20%) to the standard rat chow. The compositions of the diets are shown in table I.

Dates Fiber Extraction: Dates *(Phoenix dactyliferous)* fruits (Suk'ai Date) in Tamer stage (full ripe dry stage) were obtained from (Al-Kharj area, KSA), dates fibers were extracted in Al-Hassa Food Industries, Extraction Unit. Date fiber was extracted from the tissues of dates fruit by water extraction, as described by Prakongpan et al.[20] The dates were soaked in water (100gm / 300ml) at 60°C for 2 hr, the insoluble material was then recovered by centrifugation (4000 rpm) with running water. The residue was re-extracted and re-centrifuged with running water, then the residue of dates fibers were dried at 50C° and were used in this experiment.

Analysis of Dates Fiber: The dates fiber were analyzed at Inspection Diagnostics Consultation Laboratories (IDAC), Riyadh, KSA, to estimate their ingredients, using the procedure of the Association of Official Analytical Chemists methods. The result of the analysis shows that total dietary fiber was 79.05%, insoluble dietary fiber was 76.57% and soluble dietary fiber was 2.48%.

Experimental Design: After one week adaptation period, the Sprague-Dawley (SD) rats were divided randomly into eight groups of 8-10 rats each (Table II). Group 1 and group 5 served as control groups for non-diabetic & diabetic rats respectively. The rats were fed respective diets for a period of 24 days. They were housed individually with free access to the specified diets and drinking water in a room with controlled temperature (22-23C°); on a 12-h light-dark cycle, and 40-45% relative humidity. Body weight, food and water intake were recorded on alternative days during the experimental period.

At day 0, 10, 21 and 24, rats were deprived food overnight and anesthetized with an intramuscular injection at 60 mg/kg body weight of ketamine (HKMA pharmaceuticals Amman, Jordan), blood samples (1 ml) were collected into plain tubes by cardiac puncture. The blood samples were centrifuged at 4°C for 5 min at 3500 rpm, the separated serum was used for further biochemical analysis. For the oral glucose tolerance test (OGTT), the rats were fasted overnight and then they was administered with the glucose solution (0.3 g/100 g body weight) oro-gastricaly using a catheter tube (Vygon–Ecouen, France) and the blood samples were collected periodically at 0, 60, 120, and 180 minutes(min).

Determination of Body Weight and Biochemical Analysis

The body weights of the animals were measured using a top loader weighing balance. The biochemical analysis was carried out at the Central Laboratory and Blood bank, Ministry of Health Riyadh, Saudi Arabia. The assays were for serum glucose (fasting and OGTT) and insulin.

Blood Glucose Level

Blood sample was obtained by cardiac puncture and their fasting serum glucose was measured with an automatic analyzer (Hitachi 917, Roche modular) using commercially available enzymatic photometrical reagents by UV Test.[21] The animals were fasted for a period of 16 hours before their blood glucose level was measured.

Serum Insulin Concentration: Serum insulin concentration was measured by the Rat Insulin Enzyme immunoassay, Biotrak (EIA) system using RPN 2567 rat insulin EIA kit (Amersham Biosciences, UK) and Universal Microplate Reader Bio-Tek instrument inc USA model ELX 800.[22]

Statistical Analysis

Data were analyzed using Statistical Package for Social and Science (SPSS) program (version 10.0). Nonparametric statistics (Mann-Whitney test, and Kruskal-Wallis test) were used to compare between groups. The results were presented as mean, standard deviation and median with the level of significance at P value < 0.05.

RESULTS

The effect of dates fibers in non-diabetic and diabetic groups is presented in table III. The weight of the non-DM groups (GP1, GP2, GP3 and GP4) increased significantly throughout the entire study. Within each DM Group, the weight of DM groups (5 & 8) insignificantly increased to a lesser extent, meanwhile the weight of GP6 and GP7 decreased, reaching a significant level for GP6. Comparing each diabetic group to its counterpart DM group with the same concentration at specific days (0, 10, 21), mostly all DM groups showed a significant lower weights than their counterparts.

The effect of dates fibers on average food intake in non-diabetic and diabetic groups was shown in table IV. Throughout the study there was a significant difference in the average food intake (gm/rat/day) between the DM and Non-DM groups. The DM groups consumed significantly larger amount of food (1.3-1.5 times) as compared to respective non DM groups. Comparing the non-DM groups within each other, rats in GP4 consumed significantly the largest amount of food. While within the DM groups, the control group (GP5) consumed significantly the largest amount of food.

No significant difference was observed in the amount of water consumed by rats within non-DM or DM groups (table V), however the rats in the DM groups consumed larger amounts of water almost ≈ 400% as compared to the amount of water consumed by non-DM rats, the difference was highly significant.

Table VI shows the levels of serum glucose in non-diabetic and diabetic groups. There were no significant changes in the level of serum glucose within each of the non DM rats, except in GP2 where the level of serum glucose decreased significantly. In the DM groups, the level of serum glucose increased significantly in the control group (GP1), however the increment in the other DM groups fed on normal chow with addition of dates dietary fiber did not reach significant level. It was noticed that groups 6 and 7showed a decrease at the tenth day then increase at the twenty one day.

The DDF had lowered the level of serum insulin in non-DM groups as compared with respective their control group (GP1) but the decrements did not reach significant levels (table VII). In all DM groups, an insignificant decrease in insulin level was found in all groups by the twenty one day.

In both groups- non DM and DM rats (table VIII), the levels of serum glucose after the OGTT increased at 60 ad 120 minutes, then decrease at 180 minutes. The changes were highly significant for the non-DM groups (GP1, GP2, GP4). The levels of serum glucose after the test of OGTT were much higher in the DM groups (5, 6, 7 and 8) than in non-DM groups; the difference reached significant levels for groups with added DDF as compared to their control.

Table I: Composition of experimental diets

Ingredients	Control/control diet (g/kg)
Corn starch	465.692
Casein	140.00
Maltose and dextrin	155.00
Sucrose	100.00
Soybean oil	40.00
Fiber	50
Mineral mix	35.00
Vitamin mix	10.00
Choline bitartrate	2.50
Date Fiber:	
Control	0.00
5%	50.00
10%	100.00
20%	200.00

Table II: Experimental Animal Groups and Diet Regimens

Groups of Rats	Number of Rats	Diet % of DDF	Description of rats
GP1: Control	9	Standard + (0% dates fiber)	Non diabetic
GP2	8	Standard + 5% dates fiber	Non diabetic
GP3	8	Standard + 10% dates fiber	Non diabetic
GP4	9	Standard + 20% dates fiber	Non diabetic
GP5 : Diabetic Control	10	Standard + 0% dates fiber	Diabetic
GP6	10	Standard + 5% dates fiber	Diabetic
GP7	10	Standard + 10% dates fiber	Diabetic
GP8	10	Standard + 20% dates fiber	Diabetic

Table III: The effects of graded levels of dates dietary fiber on total body weight in non-diabetic and diabetic rats

Non-DM				DM				P#
Group No (DDF %)	Day	Weight (g / rat)	P* Value	Group No (DDF%)	Day	Weight (g / rat)	P*	
1 (0%)	0	238±12 (238.7)	0.000	5 (0%)	0	281.5±17 (281.5)	.431	.001
	10	274±11 (274.3)			10	286±18 (286.7)		.062
	21	323±9 (322.6)			21	291±18 (291.4)		.041
2 (5%)	0	255±11 (255.2)	0.000	6 (5%)	0	261±10.8 (261.8)	.029	.068
	10	298±11 (297.7)			10	227±11 (227.3)		.002
	21	339±9 (339.1)			21	217±44 (216.6)		.003
3 (10%)	0	277±14 (277.3)	0.012	7 (10%)	0	270±12 (270.3)	.172	.121
	10	304±13 (304.7)			10	255±14 (254.9)		.045
	21	331±11 (331.5)			21	246±18 (246.2)		.013
4 (20%)	0	283±11 (282.5)	0.001	8 (20%)	0	272±12 (272.1)	.215	.115
	10	314±12 (314.2)			10	280±14 (280.5)		.016
	21	350±10 (350.4)			21	300±18 (300.3)		.031

Mann- Whitney tests, *Kruskal Wallis test, data presented as mean with median between parentheses

Table IV: The effects of graded levels of dates dietary fiber on the daily food intake in non-diabetic and diabetic rats

Non-DM			DM			P #
Group No (DDF%)	Food (g / rat)	P*	Group No (DDF%)	Food (g / rat)	P*	
1 (0%)	19.3±.38 (19.32)	.010	5 (0%)	32.9 ±0.5 (32.85)	.002	.000
2 (5%)	19.6±.39 (19.53)		6 (5%)	25.9±1.8 (25.87)		.010
3 10%	18.5±.47 (18.48)		7 (10%)	28.3± 1 (28.31)		.000
4 (20%)	21.6±.95 (21.57)		8 (20%)	29.9± 1 (29.88)		.000

Mann- Whitney tests, *Kruskal Wallis test, data presented as mean ± SD with median between parentheses

Table V: The effects of graded level of dates dietary fiber on the daily water intake in non-diabetic and diabetic rats

Non-DM			DM			P#
Group No DDF%	Water (ml /rat)	P*	Group No DDF%	Water (ml /rat)	P*	
1 (0%)	29.26±1.4 (29.29)	.205	**5 (0%)**	124.9 ± 4.6 (124.5)	.081	.000
2 (5%)	29.7 ± 1.1 (29.74)		**6 (5%)**	128.6±11.1 (128.4)		.000
3 (10%)	26.7 ± 0.9 (26.59)		**7 (10%)**	136.6± 5.1 (136.8)		.000
4 (20%)	30.1 ± 1.2 (30.14)		**8 (20%)**	130.1± 5.1 (130.3)		.000

Mann- Whitney tests, *Kruskal Wallis test, data presented as mean ± SD with median between parentheses

Table VI: The effect of graded levels of dates dietary fiber on the serum glucose in non-diabetic and diabetic rats

Non-DM				DM			
Group No (DDF %)	**Day**	**Glucose Level (mmol/L)**	**P***	**Group No (DDF%)**	**Day**	**Glucose Level (mmol/L)**	**P***
1 (0%)	0	6.19±0.61 (6.20)	.061	5 (0%)	0	8.35±0.7 (8.33)	.029
	10	8.75±0.58 (8.77)			10	11.19±1.06 (11.16)	
	21	6.89±0.25 (6.85)			21	15.6±3.11 (15.58)	
2 (5%)	0	7.85±0.53 (7.87)	.019	6 (5%)	0	19.3±2.4 (19.19)	.070
	10	5.51±0.22 (5.53)			10	17.8±2.8 (17.82)	
	21	6.25±0.27 (6.24)			21	21.9±3.7 (21.88)	
3 (10%)	0	7.79±0.24 (7.76)	.100	7 (10%)	0	14.5±2.9 (14.52)	.052
	10	7.02±0.2 (7.01)			10	12.89±2.9 (12.85)	
	21	7.67±0.54 (7.69)			21	15.2±3.11 (15.34)	
4 (20%)	0	6.68±0.46 (6.69)	.059	8 (20%)	0	7.66±0.77 (7.67)	.102
	10	6.82±0.27 (6.81)			10	9.7±1.12 (9.72)	
	21	7.66±0.65 (7.65)			21	9.4±1.3 (9.44)	

*Kruskal Wallis test, data presented as mean ± SD with median between parentheses

Table VII: The effect of graded levels of dates dietary fiber on the serum insulin in non-diabetic and diabetic rats

Non-DM				DM			
Group No & (DDF %)	**Day**	**Insulin (mmol/L)**	**P# value**	**Group No & (DDF %)**	**Day**	**Insulin (mmol/L)**	**P#**
1 (0%)	0	1.12±0.35 (1.13)	.20	5 (0%)	0	1.36±0.24 (1.34)	.11
	21	1.54±0.94 (1.51)			21	1.25±0.16 (1.26)	
2 (5%)	1	2.16±0.7 (2.18)	.13	6 (5%)	0	0.99±0.21 (0.98)	.34
	21	1.45±0.19 (1.41)			21	0.82±0.5 (0.81)	
3 (10%)	1	1.97±0.38 (1.94)	.053	7 (10%)	0	0.94±0.31 (0.92)	.08
	21	1.56±0.26 (1.53)			21	0.85±0.37 (0.84)	
4 (20%)	0	1.63±0.6 (1.60)	.07	9 (20%)	0	1.25±0.21 (1.23)	.14
	21	1.34±0.49 (1.33)			21	0.84±0.14 (0.85)	

#Mann-Whitney test, data presented as mean ± SD with median between parentheses

Table VIII: The effect of graded levels of dates dietary fiber on the serum glucose in non-diabetic and diabetic rats after (OGTT)

Non-DM				DM			
Group No (DDF %)	**Time (min)**	**Glucose Level (mmol/L)**	**P***	**Group No (DDF %)**	**Time (min)**	**Glucose Level (mmol/L)**	**P***
1 (0%)	0	6.9±0.33 (6.88)	.000	5 (0%)	0	17.0±3.7 (16.9)	.201
	60	10.29±0.39 (10.26)			60	29.8±3.5 (20.88)	
	120	9.36±0.5 (9.34)			120	29.6±5 (29.62)	
	180	9.81±0.76 (9.79)			180	26.0±4 (25.98)	
2 (5%)	0	7.08±0.7 (7.07)	.031	6 (5%)	0	13.4±2.5 (13.35)	.000
	60	9.84±0.8 (9.79)			60	39.9±3.2 (39.85)	
	120	10.05±0.9 (10.08)			120	33.6±2.1 (33.61)	
	180	10.94±1.1 (10.90)			180	29.01±3.5 (29.04)	
3 (10%)	0	7.14±0.46 (7.13)	.055	7 (10%)	0	14.0±1.9 (14.11)	.000
	60	10.89±0.8 (10.87)			60	34.2±3.3 (34.31)	
	120	12.74±2.4 (12.69)			120	29.6±3.8 (29.64)	
	180	10.7±0.6 (10.74)			180	29.1±2.1 (29.13)	
4 (20%)	0	5.92±0.32 (5.89)	.000	8 (20%)	0	10.1±1.5 (10.08)	.001
	60	10.3±0.97 (10.34)			60	29.8±4.3 (29.77)	
	120	10.33±0.7 (10.30)			120	25.6±3.5 (25.59)	
	180	9.26±0.54 (9.17)			180	25.05±2 (25.11)	

*Kruskal Wallis test, data presented as mean ± SD with median between parentheses

DISCUSSION

This study was carried out to assess the effect of different concentration of dietary fibers derived from dates fruit [79.05% as total dietary fiber, 76.57% as insoluble fiber] on weight gain, serum glucose and insulin levels in STZ-induced diabetic and normal non diabetic rats.

In normal non-diabetic rats addition of dates dietary fiber did not hinder the weight gain. There was significant increase in weight gain in all four groups. Similarly the diabetic rats fed on normal diet (GP5) and also 20% DDF (GP8) in the present study, showed substantial increase in body weight. While in STZ-induced diabetic rats, 5% and 10% of DDF added diets caused reduction in weight (significant reduction only in 5% DDF). Numerous studies[23-25] have noted that consumption of dietary fibers in humans have an inverse relationship with weight gain. Adding α-cyclodextrin (a dietary fiber) to the diet significantly reduced weight gain in rats fed with a high fat diet relative to rats fed with the control diet.[26] Wang ZQ et al 2007, found that whereas there was no difference at the baseline of 3 types of dietary fibers-sugarcane fiber (SCF), psyllium (PSY), and cellulose (CEL), body weight gains in the PSY and SCF groups were significantly lower than in the CEL group at the end of study.[27] Dietary fiber has been previously shown to have an influence on weight gain because of its satiating effect.[28]

In general, it has been observed that diabetic rats had lower body weight throughout the experimental period, although they consumed 1.3 times more food as compared to non-diabetic rats. These results are in accordance with Madar[29] who found the growth of diabetic rats was retarded by STZ treatment and the growth was not related to diet.

Weight loss results from excessive protein and fat catabolism to compensate for the inability to use carbohydrate as fuel which is a direct consequence of both insulin insufficiency and increased activity of counter-regulatory hormones.

Diabetic rats fed diet with/without fibers remained hyperphagic throughout the present study. Previous studies revealed inconsistent results. Vachon et al [30] found that soluble fibers reduces food intake. Ramos N et al showed that consumption of a cholesterol-rich diet containing a soluble cocoa fiber product resulted in lower food intake and body weight gain in comparison with control groups consuming cholesterol-free or cholesterol-rich diets with cellulose as dietary fiber.[31] While Frias and Sgarbieri[32] mentioned that addition of cellulose in the diet causes an increase in food intake.

Diabetic groups consumed large amounts of water as compared with the amount consumed by non-diabetic rats, the difference was highly significant, the outcomes is notable because STZ-diabetic rats has been reported to result in significant urinary glucose loss and polyuria (Cameron-Smith et al).[33]

Whether the intake of high dietary fiber may improve glycemic control in individuals with type 2 diabetes has been controversial. The present results demonstrate that STZ-diabetic rats fed on high DDF (20%) diet showed a slight increase of serum glucose level as compared with the control (GP5), 5% (GP6), and 15% (GP7) DDF groups. Sigleo et al[34] reported that certain dietary fiber components may modify intestinal architecture and indicate that these structural changes may be correlated with altered functional characteristics. Madar et al[7] reported reduction in serum glucose when cotton seed dietary fibers - supplemented diets were given to type 2 diabetic subjects. Li J et al [35] showed that the intake of the barley diet (high dietary fibers diet) significantly improved the area under the plasma glucose concentration time curves, lowered the fasting plasma glucose and glycosylated hemoglobin levels. Meanwhile, Wang ZQ 2007 reported that fasting plasma glucose and insulin and areas under the curve of intraperitoneal glucose tolerance test were also significantly lower in the SCF and PSY groups than in the CEL and high fat diet groups.[27] Several studies[20,36-39] in which different types of insoluble dietary fiber were used, found a decreased level of blood glucose.

The serum glucose levels in non diabetic and diabetic rats decreased after 180 minutes post the oral glucose intubations as compared with their levels at 60-minuets, but did not reach their base–level (the fasting level). Madar[29] reported a decrease in plasma glucose level after glucose loading in diabetic rats fed soybean fiber.

In general, the findings of this study were inconsistent when comparing between effect of each concentration of DDF on both normal and diabetic rats groups and also when comparing between the groups and their control group (0% DDF). These inconsistencies might be attributed to some limitations of the study such as lack of placebo, the presence of fibers already in the constituents of the normal chow, different level of stress among rats groups, and differences in reaction of the rate groups to induction of diabetes by STZ. However, in conclusion, the study suggests the potential role of dates dietary fiber in reducing food intake, reducing or controlling weight, as well as lowering blood glucose among diabetic rats.

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