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Effect of Date Palm (*Phoenix Dactylifera*) Seed Fibers on Plasma Lipids in Rats

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Abstract. Five diets were investigated which include basal diet, cellulose diet and three defatted date seed fibers diets containing 1.5%, 2.5% and 5.2% date seed fiber. Wistar rats were randomly divided into five groups and the experiments proceeded for 27 days. The objective of this study was to investigate the effect of date palm (*Phoenix dactylifera*) seeds crude fiber on triglycerides, cholesterol and high density and low density lipoproteins in plasma lipids in rats. The results revealed that diet based on date seed fiber had good potential as a source of dietary fiber in diet. Diet contained 1.5% date seed fibers is the most appropriate because of its action in reducing LDL cholesterol, total cholesterol and triglyceride. However, such level (1.5%) of date seeds fiber had no effect in HDL cholesterol level.

Introduction

The effect of dietary fiber sources on lipid metabolism has been studied extensively in humans and in experimental animals [1-4]. Some dietary fiber feeding reduces blood cholesterol concentration in humans as well as in experimental animals [3, 5-8]. Dietary fiber has been suggested as a natural avalaible useful hypocholesterolaemic agent [9]. The effect on blood cholesterol depends on the type and quantity of dietary fiber eaten [10, 11] and some dietary fats can alter the hepatic LDL receptor activity [12]. The reduced blood cholesterol concentration usually involves a reduction in the low-density lipoprotein (LDL) cholesterol fractions whereas high-density lipoprotein (HDL) cholesterol is increased [3]. This may be of particular importance in the light of the present evidence that the occurrence of coronary heart disease is strongly related to decreased HDL cholesterol concentration [13, 14] and increased LDL cholesterol concentrations [15]. Work with experimental animals including rat, rabbit, chicken and swine have indicated that the supplementation of diets with soluble dietary fiber sources

retards the progression of atherosclerosis whereas insoluble dietary fiber do not have this effect [16, 17]. Date fruit is considered a high energy food due to its high content of sugar and rich in fibers. Date seeds powder contains about 10.1% fiber. Date seed fibers have been well placed in rhelogical and sensory evaluation test when mixed with wheat flour [18].

The objective of the present study was to investigate the effect of consuming fibers from palm dates seeds on triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol in rats.

Animals and diet preparation

Wistar male rats were obtained from animal house, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Upon arrival, animals (70-80 g) were housed. After equilibrium, animals were assigned to 5 groups according to diets by selective randomization. Every three rats were kept in one cage made of stainless steel with wire mesh bottom in a room controlled for temperature (22 ± 1^0 C), humidity (60-70 %), and 12 hrs on-off light cycle. Animals were adapted to the laboratory condition for 4 days and fed the basal diet to eliminate any effects of previous diets. For diet preparations, ingredients (other than that obtained from U.S. Biochemical Co.) were of food grade. Prepared diets were kept in plastic tight containers at 4^0 C. The diets and rats assigned in each group are shown in Table 1. Total consumption of diets 1-5 was based on AIN –93 rodent researches as shown in Table 1 [19]. Date seeds, as a by-product of date industry were obtained from Al-Qassim date factory (Riyadh, Saudi Arabia). Seeds were washed with water; air-dried, packed in polyethylene bags and kept -20^0C until analysis. Seeds were ground in Willey Mill and passed in Brabender Quadrument flour mill (Brabender Instrument, Inc., South Hacken, NJ). The flour was then defatted with food grade n-hexane (Sigma Chemicals, USA) and the proximate compositions were performed according to AOAC [20]. The proximate analysis showed that the defatted date seed flour contained 8.5 % moisture, 1.2% ash, 9.4% protein, 10.5 crude fiber, 0.6% fat and 69.8% carbohydrate. All the experimental groups were fed basal diet and diets supplemented with 5.2 % cellulose (diet-2), 5.2% date fibers (diet-3), 2.5% date fibers (diet-4) and 1.5 % date fibers (diet-5).

Table 1. Composition of test diets (g /100 g)

	Diet No.				
Ingredients	**1**	**2**	**3**	**4**	**5**
	Basal diet cellulose free	**Diet cellulose**	**Date fiber 5.2 %**	**Date fiber 2.5 %**	**Date fiber 1.5 %**
Sucrose	9.0	9.0	9.0	9.0	9.0
Cellulose	0.0	5.2	0.0	0.0	0.0
Date Fiber	0.0	0.0	5.2	2.5	1.5
Casein	10.0	10.0	10.0	10.0	10.0
Soybean Oil	5.2	5.2	5.2	5.2	5.2
Minerals Mix	3.5	3.5	3.5	3.5	3.5
Vitamins Mix	1.0	1.0	1.0	1.0	1.0
D L-Methionone	0.1	0.1	0.1	0.1	0.1
Corn Starch	71.20	66.00	66.00	68.70	69.70

Study protocol

Animals were allocated of all diets for 27 days. Fresh diets and water were provided without restriction. At the end of each 9 days experimental time, rats anesthetized with diethyl ether. Blood was withdrawn by syringe and by cardiac puncture in heparinzed tubes. Plasma was separated by centrifuging whole blood at 6000 rpm for 15 minutes at room temperature and stored at -70^{0} C for lipid analysis.

Total cholesterol contents of plasma (mmol/L) were determined enzymatically [20] using Human Kits (Human Gesellschaft-fur, Bio chemical and Diagnostica mbh Germany). The cholesterol in the HDL fraction, LDL fraction, and Triglycerides (mmol/L) were also measured enzymatically [21] with kits (Human Bio Chemical Co., USA)

Statistical Analysis

The effects of different percentage dietary fibers were determined by using analysis of variance and Duncan's new multiple range test [22, p.187-188]. A value of $P \leq 0.05$ was considered the criticism of significance. All the analyses were conducted in triplicate and results expressed as means and standard deviation (means ± SD).

Results and Discussion

Triglyceride concentrations of rat plasma are shown in Table 2. There were no significant effects of all diets on serum triglycerides of rats with time; however, the rats fed on the 1.5 % date seed fiber had significantly lower plasma triglycerides on day 9 than those on day 18 and day 27 (Table 2). The plasma triglycerides were lower in the rats fed with 2.5% and 1.5 % date seed fiber on day 9 and day 27 than those fed with 5.2 cellulose and 5.2 % date seed fiber (Table 2). However, no significant difference was found in plasma triglycerides of rats fed with 1.5 and 2.5% date seed fiber and basal diet in either day 9 or day 27. Triglyceride levels usually increase on diet high in available carbohydrates, starch and sugars [23]. Since diet 5 had more starch (69.70%) compared to the other diets, the beneficial effect of date seed fiber at level 1.5% was clear since it reduced serum triglycerides of the rats (Table 2).

Table 2. Means[1] and standard deviations of plasma triglycerides of rats (mmol/L) fed on different diets for 9, 18 and 27 days

Days / Diets	9	18	27
Basal diet	0.970 ± 0.31^{aB}	1.236 ± 0.66^{aA}	0.948 ± 0.25^{aB}
Basal + 5.2% cellulose	2.116 ± 0.73^{aA}	1.762 ± 0.88^{aA}	1.814 ± 0.73^{aA}
Basal + 5.2% date seed fiber	1.635 ± 0.75^{aA}	1.451 ± 0.87^{aA}	1.845 ± 0.70^{aA}
Basal + 2.5% date seed fibe	1.034 ± 0.50^{aB}	1.654 ± 0.97^{aA}	1.209 ± 0.27^{aB}
Basal + 1.5% date seed fiber	0.910 ± 0.38^{bB}	1.201 ± 0.21^{aA}	1.205 ± 0.15^{aB}

1 means of 3 replicates.

2. means with the same small letters are not significantly different ($p \leq 0.05$) within the same diet.; means with the same capital letters are not significantly different ($p \leq 0.05$) within the same day;

Generally, all levels of date seed fiber reduced the total cholesterol level of rat's blood, although the difference was not significant between the basal diet and the diet containing 2.5% date seed fiber (Table 3). Therefore, for economical and manufacturing purposes, 1.5% date seed fiber supplementation would be enough. It has been found that 10% replacement level of date seed powder was good in supplying dietary fiber as well as in rheological and sensory evaluation test [18].

Table 3 . Means[1] and standard deviations of plasma total cholesterol (mmol/L) for rats fed different diets for 9, 18 and 27 days

Days / Diets	9	18	27
Basal diet	1.027 ± 0.21^{bA}	1.382 ± 0.24^{aAB}	1.629 ± 0.17^{aA}
Basal + 5.2% cellulose	1.265 ± 0.16^{bA}	1.441 ± 0.18^{abA}	1.713 ± 0.27^{aA}
Basal + 5.2% date seed fiber	1.163 ± 0.16^{aA}	0.892 ± 0.06^{bB}	1.321 ± 0.09^{aB}
Basal + 2.5% date seed fibe	1.149 ± 0.23^{bA}	1.789 ± 0.53^{aA}	1.498 ± 0.18^{abAB}
Basal + 1.5% date seed fiber	1.262 ± 0.18^{aA}	1.249 ± 0.21^{aA}	1.255 ± 0.26^{aB}

1 means of 3 replicates.
2. means with the same small letters are not significantly different ($p \leq 0.05$) within the same diet.; means with the same capital letters are not significantly different ($p \leq 0.05$) within the same day;

Table 4 shows the effect of different levels of date seed fiber on LDL cholesterol of rat blood plasma. There were no significant differences in LDL cholesterol in rat blood plasma fed all diets over time except a reduction in LDL cholesterol in rats fed diets contained 2.5% date seed fiber on the 9th day. On the other hand, LDL cholesterol was lower in the rats fed the all levels of date seed fiber in day 9 and day 27, although the differences were not significant between the basal diet and the diets contained the all levels of date seed fiber both in day 9 and day 27. However, date seed fiber at all levels reduced significantly ($p \leq 0.05$) the LDL cholesterol in rats compared to those fed the diet containing the cellulose especially at long term feeding (27 days in this study).

Generally, all diets had no effect on HDL cholesterol over time (Table 5). Nevertheless, HDL cholesterol increased significantly ($p \leq 0.05$) in rats fed cellulose and all levels of date seed fiber compared to those fed basal diet in day 9. Rats fed diet containing 2.5% date seed fiber had significantly higher HDL cholesterol than those fed the other diets in day 18. The trend in day 27 showed a significant differences between diet 4 and diet 5 with the former (2.5% date seed fiber) having an increase in HDL in rats blood compared to diet 5 (1.5% date seed fiber). Therefore, date seed fiber at level 1.5% may not have effect on HDL cholesterol in long term feeding. Fibers usually control TC and LDL and HDL by reducing the absorption through increasing the thickness of the unstirred layer and reducing transit time in the gut [24]. Furthermore, it accomplishes that by increasing bile acid excretion in the feces and increasing the

conversion of cholesterol to bile acid. Fiber plays a role also in reducing cholesterol synthesis in the liver and small intestine indirectly by inhibiting the enzyme Hydroxy Methyl Glutaryl-CoA-reductase [24].

Table 4 . Means[1] and standard deviations of plasma LDL cholesterol (mmol/L) for rats fed on different diets for 9, 18 and 27 days

Days / Diets	9	18	27
Basal diet	0.324 ± 0.25^{aAB}	0.414 ± 0.28^{aA}	0.498 ± 0.22^{aAB}
Basal + 5.2% cellulose	0.404 ± 0.27^{aA}	0.552 ± 0.57^{aA}	0.711 ± 0.22^{aA}
Basal + 5.2% date seed fiber	0.292 ± 0.13^{aAB}	0.285 ± 0.09^{aA}	0.366 ± 0.17^{aB}
Basal + 2.5% date seed fibe	0.124 ± 0.13^{aB}	0.311 ± 0.23^{aA}	0.233 ± 0.23^{aB}
Basal + 1.5% date seed fiber	0.137 ± 0.09^{bB}	0.320 ± 0.12^{aA}	0.340 ± 0.19^{aB}

1 means of 3 replicates.
2. means with the same small letters are not significantly different ($p \leq 0.05$) within the same diet.; means with the same capital letters are not significantly different ($p \leq 0.05$) within the same day;

Table 5 . Means[1] and standard deviations of plasma HDL (mmol/L) of rats fed different diets for 9, 18 and 27 days

Days / Diets	9	18	27
Basal diet	0.430 ± 0.22^{bB}	1.024 ± 0.20^{aB}	1.127 ± 0.18^{aAB}
Basal + 5.2% cellulose	1.009 ± 0.14^{aA}	0.911 ± 0.18^{aBC}	1.002 ± 0.23^{aAB}
Basal + 5.2% date seed fiber	0.871 ± 0.09^{aA}	0.606 ± 0.02^{bC}	0.955 ± 0.17^{aAB}
Basal + 2.5% date seed fibe	1.025 ± 0.23^{bA}	1.478 ± 0.37^{aA}	1.265 ± 0.15^{abA}
Basal + 1.5% date seed fiber	1.125 ± 0.12^{aA}	1.05 ± 0.15^{aB}	0.915 ± 0.41^{aB}

1 means of 3 replicates.
2. means with the same small letters are not significantly different ($p \leq 0.05$) within the same diet.; means with the same capital letters are not significantly different ($p \leq 0.05$) within the same day;

The findings of this study suggest that diet based on date seed fiber had good potential as a source of dietary fiber in diet. The beneficial effect of raw seeds, containing good source of fiber have been well established by various experimental studies [24, 25]. Diets that are based on 1.5% date seed fiber is the most appropriate because of its action in reducing LDL, TC and TG.

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تأثير ألياف نوى التمر على مؤشرات الجلسريدات الثلاثية والكولسترول والليبوبروتينات في الفئران

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ملخص البحث. استخدم في هذه الدراسة خمسة أنواع من الوجبات والتي احتوت على ١,٥% و ٢,٥% و ٥,٢% ألياف خام من نوى التمر ووجبة مرجعية (basal diet) ووجبة تحتوي على ٥,٢% سيليلوز. تم توزيع الفئران على هذه الوجبات بشكل عشوائي واستغرقت التجربة ٢٧ يوماً. وهي تهدف لدراسة تأثير ألياف نوى تمر النخيل *(Phoenix dactylifera)* على مؤشرات الجلسريدات الثلاثية والكوليسترول الكلي وكوليسترول كل من الليبوبروتينات عالية الكثافة والليبوبروتينات منخفضة الكثافة في بلازما الفئران. بينت النتائج أن الوجبات التي تحتوي على ألياف نوى التمر من الممكن أن تكون مصدراً جيداً للألياف. الوجبة التي احتوت على ١,٥% من ألياف النوى كانت الأنسب من حيث قدرتها على خفض كوليسترول الليبوبروتينات المنخفضة الكثافة والكوليسترول الكلي والجلسريدات الثلاثية. ولم يكن هناك تأثير معنوي لهذه الوجبة على كوليسترول الليبوبروتينات المرتفعة الكثافة.