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Studies on date waste dietary fibers as hypolipidemic agent in rats

Untersuchungen zu diätetischen Fasern von Dattelabfällen als hypolipidämischer Agent bei Ratten

Summary Date waste dietary fibers were examined as a hypolipidemic agent. White albino rats were fed on three experimental diets: I) high carbohydrate diet free of fiber; II) and III) diets consisted of diet I substituted with 100 g/kg of date waste dietary fibers cultured with *Endomycopsis fibuligera* at zero time and after 60 h of culturing respectively for

8 weeks. The total lipids, total cholesterol, triglycerides and phospholipids in the liver of rats given diets II and III were significantly decreased over those rats fed the control diet throughout the feeding period (8 weeks). The highest decrease in content of all these parameters was produced by diet III.

Comparing diets II and III with the control diet I, total serum lipids and low density lipoprotein cholesterol (LDL-cholesterol) were decreased by 32–48 %, while serum triglycerides and total cholesterol levels were lowered in the groups fed diets II and III by 23–35 % respectively. Concerning high density lipoprotein cholesterol (HDL-cholesterol), the decrease was only 2–6 % in rats fed diets II and III. The highest decrease level was shown in the phospholipids content (51–56 %) during all of the experimental period (8 weeks).

Zusammenfassung Albino-Ratten wurden für 8 Wochen 3 Diäten unterworfen. Diät I bestand aus nicht-faseriger hoch-halliger Kohlenhydratkostform. Diät II und Diät III enthielten zusätzlich 100 g/kg mit *Endomycopsis fibuligera* kultivierte faserige Dattelabfälle. Die

Kultivierungszeit betrug 0 (II) und 60 Stunden (III).

Die Gesamtlipide, das Gesamtcholesterol, die Triglyzeride und die Phospholipide nahmen in den Lebern der Ratten, die mit Diät II und III gefüttert wurden, deutlicher ab, als bei denjenigen, die mit Diät I gefüttert wurden.

Bei der Diät III zeigte sich die höchste Abnahme der verschiedenen Untersuchungsparametern. Vergleicht man Diät II und III mit Diät I, so ergibt sich, daß die Serumgesamtlipide und das Lipoprotein-Cholesterol niedrigerer Dichte (LDL-Cholesterol) um 32–48 % abnehmen, während Serumtriglyzeride und Gesamtcholesterol in mit Diät II und Diät III gefütterten Ratten um 23–35 % erniedrigt sind. Bei mit Diät II und III gefütterten Ratten betrug die Abnahme des HDL-Cholesterols nur 2–6 %. Phospholipide zeigten die höchste Abnahme (51–56 %) während der ganzen Versuchsperiode (8 Wochen).

Key words Dietary fibers – agro industrial wastes – serum lipids – liver lipids

Schlüsselwörter Diäterische Fasern – industrielle Agrarabfälle – Serumlipide – Leberlipide

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Introduction

In our previous work (1), studies on production of single cell protein (SCP) from non conventional cheap sources

using the yeast strain *Pichia Pinus* and extracts of industrial fruit wastes were obtained, but in this case still left some other wastes as fibers. To overcome the pollution by residues left, it was thought worthwhile to use the

wastes as is, in semi-solid state culturing for production of SCP. Jwanny et al. (2) indicated the suitability of growing *E. fibuligera* (3812) on wet date wastes, as a potential substrate for SCP production. These protein enriched date waste cultures were found to be of beneficial importance to be used and incorporated into human foods and animal feeds.

An increased dietary intake of plant fiber is now widely accepted, due to its important role in human nutrition; it was found that these dietary fibers cause lowering of food consumption and plasma lipids in persons with hyperlipidemia (3).

Also many studies have documented the lowering effect of plant fibers on serum cholesterol concentration in humans and animals, but results concerning serum triglycerides are more controversial (4–7).

Vigne et al. (8) stated that combinations of pectins and cereal brans in the diet could be useful in normalizing cholesterol and triglycerides of patients suffering from dislipidemia.

Experimental support (in animal and man) for the hypothesis that dietary fibers may be a factor protecting against atherosclerotic diseases, showing that certain gel-forming dietary fiber constituents, especially guar gum, significantly decreased plasma cholesterol concentrations (9, 10). Studies with other types of fibers (wheat bran and baggase) have shown varying effects on serum and liver cholesterol in experimental animals (11, 12).

Schneeman et al. (13) and Stanley et al. (14) found that the high density lipoprotein cholesterol (HDL-Cholesterol) and total lipoprotein concentration were not significantly altered by diet containing 20 % wheat bran, oat bran, cellulose; 5 % pectin or 10 % guar gum.

The present study illustrated the physiological role of dietary fiber of date wastes and their effects on plasma and liver lipids of rats.

Materials and methods

Date wastes

The date wastes (outershell, fibers, stones) are those left after removing the date flesh in the manufacturing of date pastes in Kaha factories in Cairo.

Date waste dietary fibers at zero time (DW₁) and after 60 h (DW₂) of semi-solid state fermentation of the yeast *Endomycopsis fibuligera* (3812) was prepared as mentioned before by Jwanny et al. (2).

Diets composition

Table 1 illustrates the composition of the three types of diets which were used for feeding male albino rats; the first diet (I) was fiber free (control), the second (II) and third (III) diets contain diet I mixed throughly with DW₁ and DW₂ respectively.

Table 1 Composition of the basal diets

Component ^a (g/kg diet)	Control I	II	III
Starch	360.00	33.19	196.59
Sucrose	300.00	300.00	300.00
Casein	200.00	174.85	60.03
Maize oil	80.00	48.52	25.61
Mineral mixture ^b	40.00	40.00	40.00
Vitamine mixture ^b	20.00	20.00	20.00
DW ₁ ^c	–	383.40	–
DW ₂ ^d	–	–	357.80

^a Johnson and Gee (15).

^b Motzok et al. (16).

^c DW₁: Dry date waste cultures of *E. fibuligera* at zero time of fermentation 383.40 g/kg containing 100 g dietary fibers + 226.59 g starch + 25.15 g protein + 31.48 g lipid.

^d DW₂: Dry date waste cultures of *E. fibuligera* after 60 h fermentation 357.8 g/kg containing 100 g dietary fibers + 63.44 g starch + 139.79 g protein + 54.39 g lipid. Jwanny et al. (2). Starch, sucrose, maize oil are purchased from a local market. Casein from Riedel de Haen AG-D-3016, vitamine and mineral components from BDH.

Animals used and physiological assay

84 male albino rats, 60 days old, weighing 96–111 g, were divided into three groups of 28 rats each, maintained in individual cages, and were fed on one of the three diets (I, II, III) for a period of 8 weeks. Food and H₂O were given ad libitum.

At the end of every 2 weeks, rats were fasted 16 h, then blood samples were drawn from the retroorbital venous plexus with heparinized capillary tubes. Serum was separated for chemical analyses of total lipids (17), total cholesterol (18), high and low density lipoprotein cholesterol (19), phospholipids (20), triglycerides (21) and non-esterified fatty acids (22). All assays except NEF were done by Boehringer-Mannheim Kit.

Weighed samples of the removed liver of 7 rats separately from each group were lipid extracted (23) after 2, 4, 6 and 8 weeks.

In the lipid extracts, total lipid (17), total cholesterol (18), phospholipids (20) and triglycerides (21) were determined. The data of the rats fed the test diets were compared with those of the control group. Statistical analysis was performed by Student's *t*-test (24).

Results and discussion

It is clear from the results (Table 2) that the total lipids, total cholesterol, triglycerides and phospholipids in the liver of rats given diets (II, III) were significantly decreased than those rats given diet I throughout the feeding period (8 weeks). The highest decrease in all these parameters was produced by diet III. The decrease effects on lipid constituents in rat liver can be fully attributed to the presence of date waste dietary fiber in the diets

Table 2 Total lipids, cholesterol, triglycerides and phospholipids levels (mg/g): expressed on a wet weight basis in liver of white rats fed on three different diets (I, II, III)

(Mean values for 7 rats/each group)																
Diet	Total lipids (mg/g)				Cholesterol (mg/g)				Triglycerides (mg/g)				Phospholipids (mg/g)			
	Weeks				Weeks				Weeks				Weeks			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
Control I																
Mean	14.05	14.86	14.45	14.01	1.19	1.30	1.24	1.21	4.25	4.33	4.20	3.98	8.64	8.85	8.39	8.65
± S.E.	±0.34	±1.62	±0.95	±0.65	±0.08	±0.20	±0.11	±0.05	±0.27	±0.75	±0.57	±0.34	±0.30	±0.81	±0.69	±0.33
t ₃	0.00	0.49 ^a	0.40 ^a	0.06 ^a	0.00	0.51 ^a	0.37 ^a	0.21 ^a	0.00	0.10 ^a	0.08 ^a	0.62 ^a	0.00	0.22 ^a	0.33 ^a	0.02 ^a
Diet II																
Mean	10.51	10.57	10.19	10.00	0.88	0.90	0.87	0.78	3.13	2.24	2.39	2.45	7.23	7.50	6.47	6.77
± S.E.	±0.80	±0.53	±0.32	±0.40	±0.08	±0.05	±0.02	±0.01	±0.31	±0.12	±0.08	±0.13	±0.12	±0.44	±0.30	±0.34
t ₁	4.07 ^c	2.52 ^b	4.25 ^c	5.25 ^c	2.74 ^b	1.94 ^b	3.31 ^c	8.43 ^c	2.72 ^b	2.75 ^b	3.15 ^c	4.20 ^c	4.41 ^c	1.47 ^a	2.55 ^b	3.97 ^c
t ₃	0.00	0.06 ^a	0.37 ^a	0.57 ^a	0.00	0.21 ^a	0.12 ^a	1.24 ^a	0.00	2.68 ^b	2.31 ^b	2.02 ^b	0.00	0.59 ^a	2.35 ^b	1.28 ^a
Diet III																
Mean	8.98	9.51	9.21	8.88	0.65	0.67	0.59	0.48	1.98	2.05	1.91	1.70	6.25	6.67	6.53	5.26
± S.E.	±0.40	±0.53	±0.46	±0.28	±0.07	±0.04	±0.03	±0.02	±0.22	±0.14	±0.07	±0.08	±0.11	±0.46	±0.53	±0.22
t ₁	9.66 ^c	3.14 ^b	5.05 ^c	7.25 ^c	5.08 ^c	3.09 ^c	5.70 ^c	13.56 ^c	6.52 ^c	2.99 ^c	5.03 ^c	6.53 ^c	6.96 ^c	2.34 ^c	3.14 ^c	6.03 ^c
t ₂	1.71	1.41 ^a	1.91 ^a	2.29 ^b	2.16 ^b	3.59 ^c	7.77 ^c	13.42 ^c	3.03 ^b	1.03	4.52 ^c	4.91 ^c	6.02 ^c	1.30 ^a	0.10 ^a	1.26 ^a
t ₃	0.00	0.80 ^a	0.23 ^a	0.21 ^a	0.00	0.25 ^a	0.79 ^a	2.34 ^b	0.00	0.27 ^a	0.30 ^a	1.20 ^a	0.00	0.89 ^a	0.52 ^a	0.04 ^a

t₁ Significant test for DW₁ and DW₂ to control

t₂ Significant test for DW₂ to DW₁

t₃ Significant test for different weeks

Values significantly differ from the control:

^a insignificant p > 0.05

^b significant p < 0.05

^c highly significant p < 0.01

(II, III), as other food components were kept constant and the energy intake was equal for each of the three diet groups.

Once again, diet III, after 8 weeks, showed either highly significant decrease in cholesterol and triglycerides or significant decrease in liver total lipids compared with rats fed diet II. This may be explained by the presence of different amounts of monosaccharides (mannose, rhamnose and ribose) in the non-cellulosic polysaccharides of dietary fiber present in diet III which are not found in dietary fiber present in diet II due to the fermentation effect (25, 26).

These results are in accordance with those (6, 7, 26–28) who found that dietary fiber reduce the levels of total lipids, cholesterol, triglycerides and phospholipids in rat liver, but contradict those results which indicated that dietary fiber (alfalfa) supplemented to the diet elevated the levels of these parameters (12, 29–31). It was found (6, 32) that soy and wheat that contain less water-soluble fiber have a cholesterol-lowering effect and it was concluded that the use of dietary fiber in the diet of a hyperlipidemic subject could improve the lipid and carbohydrate metabolism.

Table 3 Dietary fiber components of non-starch polysaccharides (NSP) in yeast semi-solid state cultures at zero time (DW₁) and after 60 h (DW₂) fermentation

DW ₁ composition g/100g dry wt.									DW ₂ composition g/100g dry wt.								
Non-Starch polysaccharide components	dry weight g %	Cellulose	Non-cellulosic polysaccharide						dry weight g %	Cellulose	Non-cellulosic polysaccharide						
			gluc.	man.	rham.	arab.	xylose	ribose			gluc.	man.	rham.	arab.	xylose	ribose	uronic acid
Sol. NSP	20.78	—	15.28	trace	trace	1.20	3.00	trace	21.06	—	5.14	2.52	1.75	2.43	2.50	1.27	5.45
Insol. NSP	8.01	4.50	trace	trace	trace	1.66	1.5	trace	11.22	3.50	2.65	1.47	0.43	1.26	1.20	0.46	0.25
T. NSP	28.79	4.50	15.28	trace	trace	2.86	4.5	trace	32.28	3.50	7.79	3.99	2.18	3.69	3.70	1.73	5.70

Results in Table 3 (25) indicated the difference in types and amounts of dietary fibers of date waste at zero time and after 60 h of fermentation that contain soluble and insoluble cellulosic and non-cellulosic polysaccharides in a mixed type (28.8, 32.3 g %) respectively. Generally rats fed on the two diets (II, III) with date wastes dietary fibers exhibited highly significant decrease

in sera total lipids ranging from (33–48 %), total cholesterol (24–35 %), HDL-cholesterol (2–6 %), LDL-cholesterol (32–45 %), triglycerides (23–33 %), and phospholipids (51–56 %) respectively than those in the sera of rats fed the control diet (I) (Table 4). The same results have already been observed in sera of rats fed with pectin (8, 26, 33–36).

Table 4 Total lipids, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, phospholipids and non-esterified fatty acids levels in serum of white rats fed on three different diets (I, II, III)

Mean values for 7 rats/each group

Diet	Total lipids (mg/dl)								HDL-cholesterol (mg/dl)								LDL-cholesterol (mg/dl)								Triglycerides (mg/dl)								Phospholipids (mg/dl)								Non-esterified fatty acids (μEq/dl)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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t₁ = Significant test for DW₁ and DW₂ to control

t₂ = Significant test for DW₂ and DW₁

t₃ = Significant test for different weeks

a = Values significantly differ from the control:

b = insignificant p > 0.05

c = significant p < 0.05

c = highly significant p < 0.01

The present study shows that date waste dietary fiber decreases LDL-cholesterol concentrations (32–45 %) and, consequently, the LDL:HDL-cholesterol ratio decreased from 2.90 for rats fed control diet (I) to 2.08, 1.70 for rats fed diets II and III respectively; also the total cholesterol:HDL-cholesterol ratio greatly decreased from 5.40 for rats fed the control diet to 4.20 and 3.70 for rats fed diets II and III respectively.

A positive correlation exists between the incidence of coronary atherosclerosis and plasma concentration of LDL-cholesterol, which act as cardiovascular risk factor (37), so the significant decrease in LDL-cholesterol levels (32–45 %) in sera of rats given diets II and III means that date waste dietary fibers can have an effect on lowering the incidence of coronary atherosclerosis, and reducing in risk factors for cardiovascular disease. These results agree with most studies (30, 35, 38) in which oat and pectin were added to the diets.

In spite of the fact that the HDL-cholesterol carries a substantial proportion of the total plasma cholesterol, the present results (Table 4) indicate that the decrease in HDL-cholesterol percentage (2–6 %) is higher than the lowering percentage (32–45 %) of LDL-cholesterol in total plasma cholesterol of rats given diets II and III respectively than in those rats given the control diet I. These findings are consistent with those studies (8, 11) in which rats were fed wheat bran, and agree with results that showed guar gum to have a slight lowering effect of both total and HDL-cholesterol levels.

These effects of date waste dietary fibers might be due

to induced changes in the viscosity of intestinal contents and/or altered ultrastructure and some functions of the intestinal mucosa. These combined influences might impair or delay the intestinal absorption of cholesterol and fat and, consequently, the decrease in intestinal lipid biosynthesis was achieved (34, 39, 40). This might in turn decrease the liver cholesterol and triglycerides accumulation and promote beneficial changes in serum lipids and lipoprotein pattern as observed in the present study.

Also, results in Table 4 showed highly significant decrease in the level of NEFA in serum of white rats fed diets II and III as compared to those fed the fiber-free control diet throughout the 8 weeks feeding. Diet III showed highly significant decrease in NEFA level as compared with diet II. This lowering effect may be related to the decrease in cholesterol concentration which affect NEFA levels because decrease of serum rate of free fatty acid is known to decrease the triglycerides output from the liver into the circulation (41, 42). The chief emphasis in the present investigation is that the prolonged decrease of serum NEFA and cholesterol levels by date waste dietary fibers (DW1 and DW2) may be an important factor in the decreased incidence of coronary atherosclerosis.

Accordingly, it can be concluded that the use of date waste dietary fiber in the diet (100 g/kg diet) in combination with *E. fibuligera* cultures have a great loss in lipid components in serum and liver of rats and could be used as a protective factor against atherosclerosis, high blood pressure, coronary heart disease and obesity.

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