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Food Chemistry 67 (1999) 281–286

Food Chemistry

www.elsevier.com/locate/foodchem

Lipid-lowering action of pectin from *Cucumis sativus*

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Received 11 November 1998; received in revised form 13 May 1999; accepted 13 May 1999

Abstract

The oral administration of the pectin extracted from the fruit of *Cucumis sativus* at a dose of 5 g kg^{-1} body weight day^{-1} showed significant hypolipidemic action in normal as well as cholesterol-fed experimental animals. Concentrations of cholesterol, triglycerides, phospholipids and free fatty acids were found to be significantly reduced in the serum and tissues of experimental animals. Activity of HMG CoA reductase was found to be enhanced. Pectin administration decreased the activities of glucose-6-phosphate dehydrogenase and malate dehydrogenase while it increased the activities of lipoprotein lipase and plasma LCAT. Incorporation of labelled ^{14}C acetate into free cholesterol was significantly higher in the liver of pectin-treated rats. Concentrations of bile acids (hepatic and fecal) and fecal neutral sterols showed significant increases in the pectin-administered groups.

Keywords: *Cucumis sativus*; Hypolipidemic; Pectin; HMG CoA reductase; Glucose-6-phosphate dehydrogenase; Malate dehydrogenase; Lipoprotein lipase and LCAT

1. Introduction

Hypolipidemic activities of pectins from various sources are well documented (Bobek & Chrovathova, 1984; Hexberg, Hexberg, Willumson & Berge, 1994; Judd & Truswell, 1985). Some important parameters which influence the activity of any active component are the diverse procedures adopted for isolation of the active principle, the diversity of sources selected, variation in storage time, physicochemical conditions that (directly or indirectly) produce alteration in the composition of the material and differences in selecting the dose of the material. Supplementing the diet with certain fibres can be regarded as an important therapeutic measure in the treatment of diabetic and obese patients. However, inclusion of pectin-rich fruits, vegetables, or cereals in the diet can supplement adequate amounts of pectin required for the beneficial effects. Dietary incorporation of pectin appears to affect several metabolic and digestive processes; those of principal interest are the effects on glucose and cholesterol levels. Hypoglycemic activity of pectin from *Coccinia indica* (Prasannakumar, Sudheesh & Vijayalakshmi, 1993) has been reported. Numerous studies have shown that pectin administration reduces serum cholesterol (Larry, Kathleen, Helenbeth & Donald, 1990; Patzy & Richard, 1990; Robert, 1994). Previous studies indicate that pectins from many fruits, vegetables and cereals significantly reduce lipid levels in serum and tissues (Anderson & Chen, 1979; Kay & Truswell, 1977; Meittinen & Tarpila, 1977). The hypocholesterolemic effects of pectin were studied in weanling Sprague–Dawley rats (Judd & Truswell, 1985) and plasma cholesterol levels were significantly reduced by pectins. Most studies have shown that supplementation of pectin lowers LDL cholesterol and in some cases VLDL cholesterol rather than HDL cholesterol (Robert, 1994). In humans, total serum cholesterol was lowered by pectin supplementation without reducing HDL cholesterol levels (Durrington, Manning, Button & Hartog, 1976; Jenkins, Reynolds, Leeds, Waller & Cummings, 1979). Since the fruit of *Cucumis sativus* is widely used as a vegetable all over India, we have undertaken investigations on the effect of pectin extracted from it, on lipid metabolism.

2. Materials and methods

All the biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St. Louis, MO, USA, and the chemicals from BDH India.

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0308-8146/99/$ - see front matter
PII: S0308-8146(99)00135-1

Male albino rats (Sprague–Dawley strain), weighing 80–120 g, bred in our animal house, maintained at 28–32°C, and having a 12 h alternate light and dark cycle, were used for these studies. The institutional guide for use and care of laboratory animals was followed throughout the study. A standard pellet diet (Gold-Mohur rat feed) and tap water were given ad libitum for 45 and 90 days in the first and second experiments, respectively.

Pectin was extracted from cucumber fruit by EDTA extraction procedure (Arslan, 1995). *Cucumis sativus* pulp (1 g) was mixed with 100 ml of 0.01 N HCl (pH 3.5) and 100 ml 0.5% (w/v) of EDTA. The mixture was mechanically stirred for 60 min at 90°C, cooled to 25°C; pH was adjusted to hydrolyse the proteins present in the extract. The extract was incubated overnight at 37°C and the liquid phase separated from the solid mass by filtering. Pectin was coagulated with 4 volumes of ethanol, centrifuged for 30 min. Pectin coagulate was washed twice with 45% ethanol, centrifuged for 30 min and dried.

2.1. Experiment I

The rats were divided into 2 groups with 12 rats in each group. Group I served as the control while group II received 5 g cucumber pectin kg^{-1} body weight day^{-1} which was found to be the most effective dose (Sudheesh, 1996). At the end of the experiment, rats were starved for 18 h and sacrificed. The tissues were removed to ice-cold containers for various estimations. For the estimation of fecal bile acids, feces were collected by transferring the animals to metabolic cages for 24 h period. Standard procedures were adopted for analysing different parameters, as described previously (Valsa, Usha Kumari & Vijayalakshmi, 1995).

2.2. Experiment II

The rats were divided into 2 groups with 12 rats in each group. Group I was treated as control and group II were supplied 5 g Cucumber pectin kg^{-1} body weight day^{-1}. The rats were supplied with atherogenic diet and water ad libitum. The composition of the diet supplied was as described by Gomathy, Vijayalakshmi & Kurup (1989). After the completion of the period, rats were sacrificed and tissues were removed to ice-cold containers for various estimations. Standard procedures were adopted for analysing different parameters as described previously (Valsa et al., 1995). Statistical significance was calculated using Students *t*-test (Bennet & Franklin, 1967).

2.3. In vivo studies on lipids

The rats deprived of food overnight were injected intraperitoneally with 0.5 ml solution of 58.5 mmol (10 μci) of 1,2-^{14}C sodium acetate at 09:00; 3 h after injection the rats were killed by decapitation. The liver was quickly dissected out. The tissue was gently blotted and homogenised in 80% aqueous ethanol. After saponification with 10 N NaOH for 1 h lipids were extracted with petroleum ether. Cholesterol was separated by TLC over silica gel (silica gel G; solvent system—hexane:ether:acetic acid 80:20:1 v/v/v) and activity counted in a Packard Priya liquid scintillation counter. The scintillant fluid was 2,5 diphenyl oxazole (PPO) 6 g and 1/4-bis (2–15 phenyl oxazoly1) benzene (POPOP) 0.2 g/l of toluene.

3. Results

3.1. Results of experiment I

Food intake and body weight did not show any significant variation among the two groups of animals. Concentration of cholesterol was lowered significantly in the serum, liver, aorta, heart and kidney of group II animals ($p < 0.01$). Concentration of triglycerides was lowered significantly in the liver and serum of pectin-treated animals ($p < 0.01$). Phospholipid levels showed significant reduction in the liver, kidney, heart and aorta of experimental animals as compared to the control group animals ($p < 0.01$). Free fatty acid levels were significantly reduced in liver, serum and heart of group II animals (Fig. 1).

Activity of the enzyme, HMG CoA reductase (EC.1.1.1.34), showed enhancement in the pectin-administered animals (Fig. 2), indicating higher rate of cholesterogenesis. Activities of the enzymes glucose-6-phosphate dehydrogenase (EC.1.1.1.49) and malate dehydrogenase (EC.1.1.1.40) showed significant reduction ($p < 0.01$) in the pectin-administered animals (Table 1), denoting a lower rate of lipogenesis. Activities of lipoprotein lipase (LPL; EC.3.1.1.3) and plasma LCAT (LCAT: EC. 2.3.1.3) were significantly increased ($p < 0.01$) in the pectin-treated animals (Table 1). The incorporation of labelled [^{14}C] acetate into free cholesterol was significantly higher in the liver while it was lower in the serum of experimental group animals ($p < 0.01$). The ester cholesterol, triglycerides and phospholipids showed a significantly decreased incorporation of [^{14}C] acetate ($p < 0.01$) in both serum and liver of group II animals (Table 2). Concentration of hepatic and fecal bile acids and fecal neutral sterols showed significant elevation ($p < 0.01$) in the group II animals (Table 3).

3.2. Results of experiment II

Food intake and body weight did not show any significant variation between the two groups of animals. The concentration of cholesterol was decreased significantly

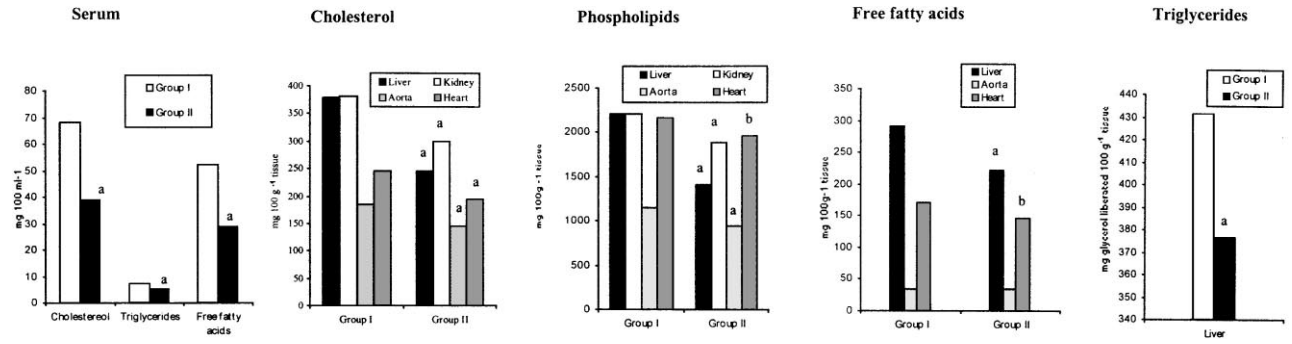

Fig. 1. Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on concentrations of cholesterol, phospholipids, free fatty acids and triglycerides in serum and tissues of rats fed cholesterol-free diet. Average of the values of 12 rats in each group ± SE. Group II is compared with group I; a = $p < 0.01$, b = $0.01 < p < 0.05$ between groups I and II.

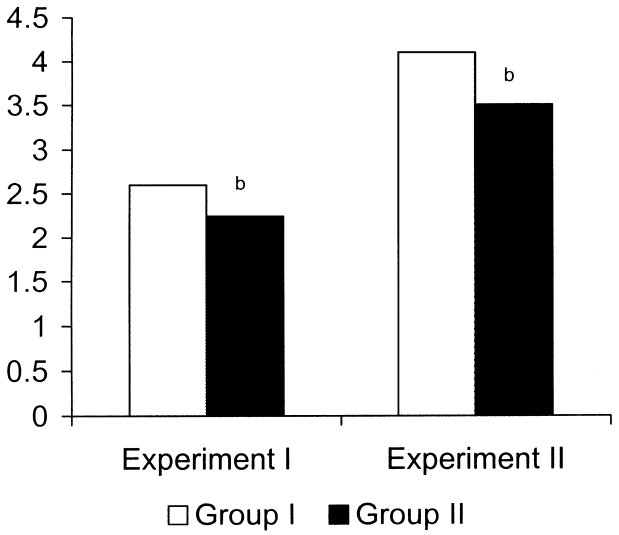

Fig. 2. Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on HMG CoA reductase activity in the liver of rats fed cholesterol-free diet (Experiment I) and cholesterol-containing diet (Experiment II). Activity is expressed as the ratio of [HMG CoA/mevalonate] i.e. the lower the ratio, the higher the activity. Values are mean ± SE for 12 rats. Group II is compared with group I; b = $0.01 < p < 0.05$ between groups I and II.

in the serum, liver, kidney and heart of experimental animals ($p < 0.01$). Triglycerides showed significant reduction in the serum of group II animals ($p < 0.01$). The concentration of phospholipids was decreased significantly in the liver and kidney of pectin-administered rats ($p < 0.01$). Free fatty acid levels also showed significant reduction in the liver and serum of pectin-treated animals ($p < 0.01$) (Fig. 3).

Activity of HMG CoA reductase was increased in the liver of group II animals (Fig. 2) as in the case of experiment I. Both glucose-6-phosphate dehydrogenase and malate dehydrogenase activities were significantly decreased ($p < 0.01$) in the pectin-adminstered rats (Table 1), showing a decreased rate of lipogenesis. Activities of enzymes like LCAT in plasma and lipoprotein lipase in the heart and adipose tissue showed significant elevation ($p < 0.01$) in the pectin-treated rats (Table 1). Concentration of hepatic bile acids, fecal bile acids and fecal neutral sterols showed significant elevation ($p < 0.01$) in the experimental group of animals as compared to the control group (Table 3).

4. Discussion

Results of the above experiments indicate that pectin from *Cucumis sativus* is highly beneficial in reducing lipid levels. Contemporary evidence supports the view that soluble fibre, such as pectin, may be a useful adjunct to the dietary management of elevated plasma cholesterol (Reddy, Watanabe & Sheinfil, 1980; Ruth, 1976; Wilson et al., 1984). It was demonstrated that prickly pear (Opuntia sp.) pectin reversed LDL receptor suppression induced by a hypercholesterolemic diet in guinea pigs (Fernandez, Lin, Trejo & McNamara, 1992). Dietary incorporation of pectin appears to affect several metabolic and digestive processes, those of principal interest being the effects on glucose absorption and cholesterol levels.

Several studies have shown that dietary supplementation with pectin increases excretion of fecal fat, sterols, and bile acids in men (Kay & Truswell, 1977; Meittinen & Tarpila, 1977). Studies on guinea pigs demonstrate that increased excretion of bile acids interrupts the enterohepatic circulation, causing an increase in liver function leading to bile acid synthesis from cholesterol (Fernandez, Trejo & McNamara, 1990). Reports are also available that pectins contribute to many physiological effects, such as lowering of cholesterol by binding to bile acids and thus increasing cholesterol catabolism (Furda, 1979; Meittenen & Tarpila, 1977). It has been reported that cholesterol absorption is decreased by pectin, as reflected in increased fecal cholesterol content (Anderson & Clark, 1986). Some reports, based on experiments in rats, suggest that endogeneously synthesized cholesterol is the preferred substrate for bile acid synthesis and would thus no longer be available to raise

Table 1
Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on the activities of lipogenic enzymes in the liver, lipoprotein lipase in the heart and adipose and plasma LCAT in rats fed cholesterol-free diet (Experiment I) and cholestorol-containing diet (Experiment II)[a]

	Experiment I		Experiment II	
Enzymes	Group I (control)	Group II (experimental)	Group I (control)	Group II (experimental)
Glucose-6-phosphate dehydrogenase[b] (units g^{-1} protein)	107 ± 4.3	69.9 ± 1.8a	1060 ± 42.4	804 ± 24.1d
Malate dehydrogenase[c] (units g^{-1} protein)	143 ± 5.7	91.9 ± 2.2a	1192 ± 47.7	475 ± 9.5a
Lipoprotein lipase[d]				
Heart	32.4 ± 0.64	58.9 ± 2.4a	21.5 ± 0.54	34.4 ± 10.3a
Adipose	156 ± 3.11	202 ± 5.6a	129 ± 2.6	187 ± 7.1a
LCAT[e]	25.6 ± 0.51	29.1 ± 1.07b	20.8 ± 0.52	37.6 ± 1.5a

[a] The values are the mean ± SE for 12 rats. Group II is compared with group I. a = $p < 0.01$ between group I and II; b = $0.01 < p < 0.05$ between groupsI and II.
[b] One unit is defined as that amount of the enzyme that causes an increase of 0.01 in optical density min^{-1} mg^{-1} protein.
[c] One unit is defined as that amount of the enzyme that causes and increase of 1 in optical density min^{-1} mg^{-1} protein.
[d] μ mol of glycerol liberated h^{-1} g^{-1} protein.
[e] Activity expressed as % increase in the ratio of ester cholesterol to free cholesterol during incubation.

Table 2
Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on in vivo incorporation of [^{14}C] acetate in rats fed cholesterol-free diet (values expressed as counts min^{-1} g^{-1} tissue)[a]

Serum and liver	Group I (control)[b]	Group II (experimental)
Serum		
Free cholesterol	720 ± 28.8	640 ± 23.1
Ester cholesterol	480 ± 19.2	285 ± 8.5a
Triglycerides	864 ± 34.6	690 ± 22.1a
Phospholipids	321 ± 12.8	280 ± 10.1b
Liver		
Free cholesterol	1242 ± 24.8	1705 ± 682.1a
Ester cholesterol	472 ± 18.8	279 ± 6.9a
Triglycerides	892 ± 35.6	591 ± 17.2a
Phospholipids	4185 ± 167.4	2134 ± 42.7a

[a] The values are the mean ± SE for 12 rats.
[b] Experimental group has been compared with control group a = $p < 0.01$ between group I and II; b = $0.01 < p < 0.05$ between groups I and II.

body cholesterol concentration (Bjorkhem & Danielson, 1975). Arjmandi et al. investigated cholesterol metabolism in rats with relevance to pectin and suggested that increase in fecal elimination of neutral sterols by ingestion of pectin is balanced by an increase in hepatic sterol synthesis (Arjmandi, Ahn, Nathani & Reeves, 1992). According to another report, pectin-fed rats exhibited significantly higher HMG CoA reductase activity than the fibre-free control group (Patzy, Barbara, & Richard, 1991). A decrease in bile acid reabsorption enhances cholesterol synthesis in the liver of rats (Lutton, 1976). The higher activity of HMG CoA reductase and higher incorporation of labelled acetate into hepatic-free cholesterol, in rats administered pectin from *Cucumis sativus*, indicate that cholesterogenesis is significantly increased in spite of the lower plasma cholesterol levels detected in these rats. However, the increased activity of the enzyme plasma LCAT, which is involved in the transport of cholesterol from the tissues to the liver for its catabolism, may be a causative factor for the significant decrease in the concentration of cholesterol in the serum and tissues of pectin-treated rats. To summarise, hypocholesterolemic activity exerted by this pectin may be due to the higher rate of degradation of cholesterol to bile acids and neutral sterols, as is evident from the higher concentrations of hepatic and fecal bile acids and fecal neutral sterols and increased removal of cholesterol from extrahepatic tissues and transportation to liver, the site of degradation, by the stimulated activity of plasma LCAT, and

Table 3
Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on concentrations[a] of the bile acids and neutral sterols in rats fed cholesterol-free diet (Experiment I) and cholesterol-containing diet (Experiment II)[b]

	Experimental I		Experimental II	
Bile acids and neutral sterols	Group I (control)	Group II (experimental)	Group I (control)	Group II (experimental)
Liver bile acids	25.4 ± 0.76	32.4 ± 1.2a	45.4 ± 1.2	54.6 ± 2.0a
Fecal bile acids	19.4 ± 0.58	30.0 ± 1.2a	63.2 ± 1.6	74.1 ± 2.6a
Fecal neutral sterols	10.6 ± 0.33	19.2 ± 0.77a	44.9 ± 1.1	55.7 ± 1.9a

[a] Values are expressed as mg 100 g^{-1} west tissue; feces mg rat^{-1} 24 h^{-1}.
[b] The values are the mean ± SE for 12 rats. Experimental group has been compared with control group a = $p < 0.01$ between groups I and II.

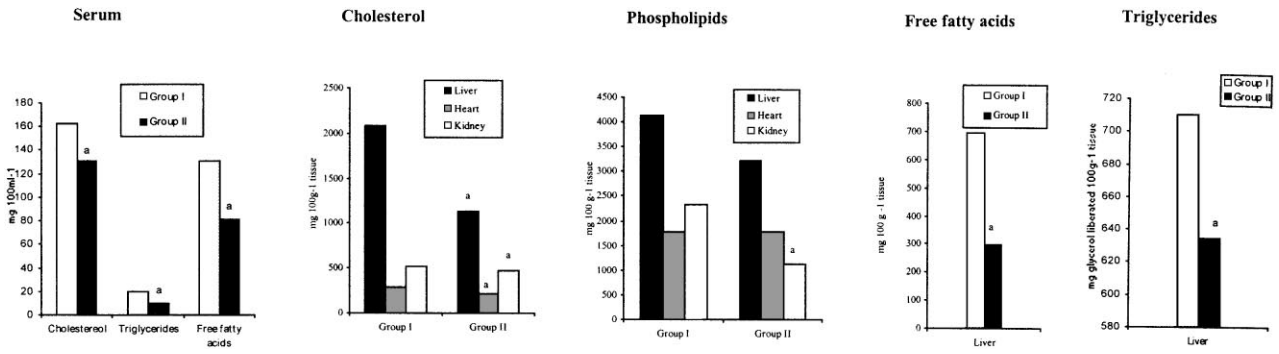

Fig. 3. Effect of pectin (5 g kg^{-1} body weight day^{-1}) from *Cucumis sativus* on concentrations of cholesterol, phospholipids, free fatty acids and triglycerides in serum and tissues of rats fed cholesterol-containing diet. Average of the values of 12 rats ± SE. Group II is compared with group I; a = $p < 0.01$, between groups I and II.

lower rate of reabsorption of cholesterol and bile acids (Kay & Truswell, 1977).

The activities of the lipogenic enzymes, glucose-6-phosphate dehydrogenase and malate dehydrogenase, in pectin-fed rats, showed significant decrease, which accounts for the lower levels of lipids in tissues. The increased activity of lipoprotein lipase (LPL) in the adipose and heart tissues may be responsible for the decreased concentration of triglycerides in serum and liver of rats administered pectin, since LPL is involved in the uptake of circulating triglyceride-rich lipoproteins (chylomicrons and VLDL) by the extra hepatic tissues for degradation (Gomathy et al., 1989).

Thus the results of these studies can be concluded in such a way that pectin from *Cucumis sativus* is an effective hypolipidemic agent at a dose of 5 g kg^{-1} body weight day^{-1}. Administration of this pectin to rats induces hypocholesterolemia and hypotriglyceridemia.

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