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Hypolipidemic principle of the inflorescence stalk of plantain (*Musa sapientum*)

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Abstract. A pectin present in the juice of the inflorescence stalk of plantain (*Musa sapientum*) has been isolated. The material contained 32·4% hexoses and 52·5% uronic acid. On administration to rats fed both cholesterol free and cholesterol diet, this material showed significant lowering of cholesterol and triglycerides in the serum, liver and aorta. There was decreased cholesterogenesis in the liver as was evident from decreased activity of hydroxymethylglutaryl coenzyme A reductase and decreased incorporation of labelled acetate into hepatic cholesterol. Hepatic bile acids showed significant increase and there was increased fecal excretion of neutral sterols and bile acids. Release of lipoproteins into the circulation was lower. The material also caused increase in the activity of lipoprotein lipase in the heart and adipose tissue and also of plasma lecithin: cholesterol acyl transferase.

Keywords. *Musa sapientum;* cholesterol; triglycerides; HMG CoA reductase; lipoprotein lipase; plasma LCAT.

Introduction

The stem (inflorescence stalk) of plantain (*Musa sapientum*) is widely used as a vegetable in south India. The juice of the stem is claimed to have beneficial effects in reducing obesity by local practitioners of naturopathy. This fact led us to study the effect of the juice of the stem on the level of lipids (cholesterol and triglycerides) in the serum and tissues in rats fed cholesterol free and atherogenic diet. It was found in these preliminary experiments that administration of the juice resulted in significant lowering of cholesterol and triglycerides in the serum and tissues in these rats. This paper describes the results of studies on the isolation of the hypolipidemic principle from the juice and the mechanism of this effect.

Materials and methods

Preliminary studies showed that a polysaccharide containing uronic acid was responsible for at least some of the activity exhibited by the juice. The following procedure was therefore adopted for its isolation.

Fresh juice of the pith of the stem of plantain was treated with cetyl pyridinium chloride (CPC) to a final concentration 1% CPC. CPC formed an insoluble complex with the polysaccharide. The complex was collected by centrifugation,

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Abbreviations used: CPC, Cetyl pyridinium chloride, TCA, trichloroacetic acid; HMG CoA, hydroxymethylglutaryl coenzyme A; VLDL, very low density lipoprotein; LDL, low density lipoprotein; LCAT, lecithin: cholesterol acyl transferase; HDL, high density lipoprotein; LPL, lipoprotein lipase.

dissolved in 3 M $MgCl_2$ solution and the polysaccharide precipitated by the addition of 4–5 volumes of ethanol. Since this material contained small amounts of protein, it was digested with papain (crystalline papain, l/3rd the dry weight of the material) in 0·1 M phosphate buffer pH 6·5, containing 0·005 M EDTA and 0·005 M cysteine for 48 h at 65 °C, with change of papain every 16 h. The papain digest was deproteinised with trichloroacetic acid (TCA) (final concentration 10%) and the supernatant dialysed till free of TCA. The polysaccharide was precipitated from the solution by 4–5 volumes of 95% ethanol containing 1-2% potassium acetate. The precipitate collected by centrifugation was dried in vacuum. The yield of this material was 1·5 g per litre of the juice.

Total uronic acid was estimated in the material by the modified procedure of Bitter and Muir (1962). Total hexose Bas estimated by the phenol-sulphuric acid method (Dubois *et al.,* 1966).

Animal experiments

Composition of cholesterol free diet (g/100 g)

Corn starch	71
Casein (vitamin and fat free)	16
Groundnut oil	8
Salt mixture	4
Vitamin mixture	1

Composition of high fat cholesterol diet (g/100 g)

Corn starch	62
Casein (vitamin and fat free)	16
Coconut oil	15
Salt mixture	4
Vitamin mixture	1
Cholesterol	2

The composition of the salt mixture and vitamin mixture has been described before (Menon and Kurup, 1976).

Rats fed cholesterol free diet: Male albino rats (Sprague–Dawley strain, weighing 80–100 g) receiving cholesterol free diet were divided into groups 1 and 2 of 12 rats each. Group 1 served as control while the group 2 was given the test material (20 mg/100 g/day orally for 45 days).

Rats fed high fat-cholesterol containing diet: In another experiment, two groups of rats (weighing 80–100 g) receiving high fat cholesterol diet were divided into groups 3 and 4 of 12 animals each. Group 3 served as control while group 4 received the test material (20 mg/100 g/day orally for 90 days).

The material dissolved in water (1 ml) was administered daily to the animals of groups 2 and 4, orally by tube as a single dose. This dose was selected, since in preliminary experiments with various doses (5, 10, 15, 20, 25 mg/100 g body weight) maximum cholesterol lowering action was observed at this level. There was no

further increase in cholesterol lowering action at higher levels. At the end of the experimental period, the rats in each group were starved overnight and killed by stunning and decapitation.

Cholesterol and triglycerides were estimated in the serum and tissues as described earlier (Leelamma *et al.,* 1978). Extraction of liver bile acids was carried out according to the procedure of Okishio *et al.* (1967) and bile acids estimated enzymatically using 3 α-hydroxysteroid dehydrogenase (Robert, 1969). Changes in *ß*-hydroxymethylglutaryl coenzyme A (HMG CoA) reductase activity (EC 1·1·34) of tissues were estimated as described by Venugopala Rao and Ramakrishnan (1975) by determining the ratio of HMG CoA: Mevalonic acid. Separation of serum lipoproteins was carried out by the procedure of Warnick and Albers (1978) and that of Burstein and Scholnick (1973). Very low density lipoprotein (VLDL) was precipitated by sodium dodecyl sulphate (final concentration 0·7%) and VLDL + low density lipoprotein (LDL) by the heparin and manganese precipitation method (final concentration heparin 0·144% and $MnCl_2$ 0·091 M). Release of lipoproteins into the circulation was studied using Triton WR 1339 as described earlier (Lakshmi Prabha *et al.,* 1988). Fecal neutral sterols and bile acids were extracted by the general procedure of Grundy *et al.* (1965). Neutral sterols and bile acids were estimated as mentioned above. Activity of lipoprotein lipase (LPL) of the heart and adipose tissue was determined according to the procedure of Krauss *et al.* (1974). Protein was determined by the method of Lowry *et al.* (1951). *In vivo* incorporation of 1, 2- [^{14}C] acetate into tissue lipids was carried out as described earlier (Molly Thomas *et al.,* 1983). Assay of plasma lecithin: cholesterol acyl transferase (LCAT) (EC 2·3·1·3), was carried out as described by Annie Abraham and Kurup (1988).

For histochemical examination, the tissues from the rats fed cholesterol free diet were removed to 10% buffered formalin. Sections were stained with heamatoxylin and eosin.

Results and discussion

The material on analysis contained 32·4% total hexoses and 52·5% uronic acid. It therefore appears to be a pectin. It is free from protein. Pectins from several sources have been reported to contain condensed tannins, which are rather difficult to remove (Fry, 1983; Selvendran *et al.,* 1985). Estimation of tannins in the material according to the procedure of Cameron *et al.* (1943) showed that it contained 3–4% of tannins.

Diet consumption was similar (10·5±1·5 g) in the rats of groups 1 and 2 and also in 3 and 4 (11·5±1·3 g). However, gain in weight was significantly lower in the rats given the material (45±1·2 and 25±l·5g in the control and experimental rats respectively in the cholesterol free diet group and 90±2·5 and 68±2·8 g respectively in the cholesterol diet group). The liver weight per 100 g body weight was however comparable in the rats of the control and experimental animals both in the cholesterol free and cholesterol diet groups (3·01±0·24 g in the cholesterol free diet group and 2·6±0·28 g in the cholesterol diet group).

Histochemical examination of tissues

Histochemical examination of the liver, heart, kidney and brain showed no

abnormality in the experimental groups. No sign of necrosis was evident indicating that the material is non-toxic at the dose used (figures 1 and 2).

Concentration of cholesterol and triglycerides in the serum and tissues

Concentration of cholesterol was significantly lower in the serum, aorta and liver in the rats given the material in both the cholesterol free and cholesterol diet groups (table 1). Triglycerides were also significantly lower in the serum and these tissues and also in the adipose tissue in the rats given the material.

Concentration of cholesterol in the serum lipoproteins

The concentration of cholesterol in VLDL, LDL and high density lipoprotein (HDL) was significantly lower in the rats given the material in both cholesterol free and cholesterol diet groups (table 2). Thus the material shows significant cholesterol and triglyceride lowering action in the serum and tissues. The hypocholesterolemic action in the serum was manifested in all the lipoprotein fractions.

Activity of HMG CoA reductase

The activity of HMG CoA reductase was significantly lower in the liver in the rats given the material in the cholesterol free diet groups (table 2). The incorporation of label into free and ester cholesterol in liver was also significantly lower. This is understandable since the activity of HMG CoA reductase generally correlates very closely with the rate of cholesterol biosynthesis. The material causes decreased cholesterogenesis in the liver of normal animals.

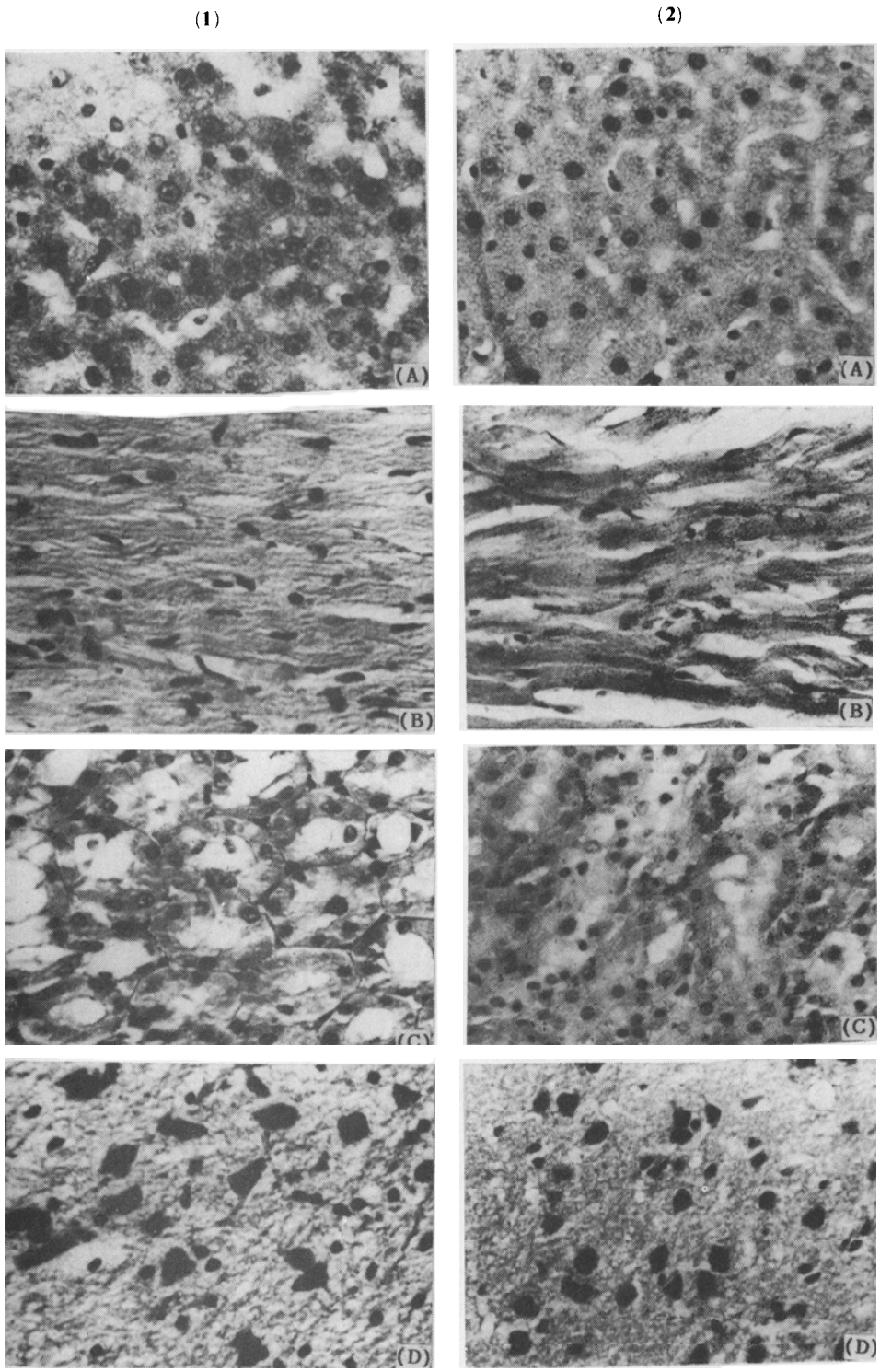
Concentration of bile acids

Concentration of total bile acids in the liver showed significant increase in the liver in the rats given the material in both cholesterol free and cholesterol diet groups (table 3). There was also increased fecal excretion of neutral sterols and bile acids in these rats. The increased concentration of hepatic bile acids may probably be due to increased hepatic degradation of cholesterol to bile acids.

Thus the hypocholesterolemic activity of the material in rats fed cholesterol free diet may be due to decreased hepatic cholesterogenesis, increased degradation of cholesterol into bile acids in the liver and increased fecal excretion of neutral sterols and bile acids. In rats fed cholesterol diet also, increased degradation of cholesterol to bile acids and fecal excretion of neutral sterols and bile acids may be responsible for the cholesterol lowering action. Cholesterogenesis in rats fed cholesterol diet was not studied.

Release of lipoproteins into the circulation

There was decreased release of lipoproteins into the circulation in the rats given the material in both the cholesterol free and cholesterol diet groups (table 4). The lower



Figures 1 and 2. Cells from liver (**A**), heart (**B**), kidney (**C**) and brain (**D**) tissues. (**1**) Control rats (× 400). (**2**) Experimental rats given material (× 400)

Table 1. Concentration of cholesterol and triglycerides in serum and tissues.

Groups	Cholesterol			Triglycerides (expressed as triglyceride glycerol)			
	Serum (mg/100 ml)	Liver (mg/100 g wet tissue)	Aorta	Serum (mg/100 ml)	Liver	Aorta (mg/100 g wet tissue)	Adipose tissue
Cholesterol free diet							
1. Control	69·0 ± 2·0	367·0 ± 12·0	187·0 ± 6·0	6·0 ± 0·0	392·0 ± 12·0	647·0 ± 20·0	5154·0 ± 164·0
2. Experimental	46·15 ± 1·02[a]	270·0 ± 9·45[a]	152·9 ± 4·8[a]	5·1 ± 0·16[a]	257·6 ± 8·24[a]	511·0 ± 22·75[a]	3910·45 ± 125·13[a]
Cholesterol diet							
3. Control	125·3 ± 4·01	1435·2 ± 50·2	387·1 ± 13·55	14·8 ± 0·47	797·9 ± 25·53	1730·3 ± 55·4	7864·52 ± 251·67
4. Experimental	99·16 ± 3·5[a]	1078·4 ± 37·7[a]	326·0 ± 11·41[a]	10·2 ± 0·33[a]	548·2 ± 17·54[a]	1216·1 ± 38·92[a]	5263·25 ± 168·4[a]

Values are the mean ± SEM for 12 rats. Group 2 has been compared with group 1 and group 4 with group 3. [a] $P < 0·01$.

Table 2. Concentration of cholesterol in the serum lipoproteins, activity of HMG CoA reductase and incorporation of label into hepatic cholesterol.

Group	Concentration of cholesterol in serum lipoproteins (mg/100 ml serum) HDL	LDL	VLDL	Activity of hepatic HMG CoA reductase (ratio of HMG CoA to mevalonate*)	Incorporation of 1,2-[^{14}C] acetate into cholesterol in liver (counts/min/g tissue) Free	Ester
Cholesterol free diet						
1. Control	51·7 ± 1·65	15·2 ± 0·49	7·3 ± 0·24	2·2 ± 0·08	1154 ± 40·39	352 ± 12·32
2. Experimental	36·1 ± 1·15[a]	7·61 ± 0·24[a]	3·12 ± 0·09[a]	2·8 ± 0·09[a]	958 ± 33·53[a]	216 ± 7·56[a]
Cholesterol diet						
3. Control	69·41 ± 2·2	41·02 ± 1·31	10·5 ± 0·34	NS	NS	NS
4. Experimental	57·23 ± 1·8[a]	21·67 ± 0·69[a]	5·34 ± 0·17[a]	NS	NS	NS

*Smaller ratio indicates higher enzyme activity.
Values are the mean ± SEM for 12 rats. Group 2 has been compared with group 1 and group 4 with group 3. [a] $P < 0·01$.
NS, Not studied.

Table 3. Concentration of bile acids in the liver and fecal excretion of neutral sterols and bile acids.

Groups	Bile acids Liver (mg/100 g tissue)	Fecal bile acids (mg/24 h/rat)	Fecal sterols
Cholesterol free diet			
1. Control	31·7 ± 1·11	29·7 ± 1·04	28·6 ± 1·2
2. Experimental	53·3 ± 1·65[a]	40·1 ± 1·44[a]	39·8 ± 1·8[a]
Cholesterol diet			
3. Control	44·6 ± 1·56	38·4 ± 1·34	64·5 ± 2·6
4. Experimental	77·5 ± 2·71[a]	66·2 ± 2·32[a]	88·9 ± 3·2[a]

Values are the mean ± SEM for 12 rats. Group 2 has been compared with group 1 and group 4 with group 3. [a] $P < 0·01$.

Table 4. Release of lipoproteins into circulation, activity of LPL and plasma LCAT.

Groups	Release of lipoproteins into circulation*	Activity of LPL (μmol of glycerol/h/g protein) Heart	Adipose	Activity of plasma LCAT (percentage increase in the ratio of ester cholesterol to free cholesterol during incubation)
Cholesterol free diet				
1. Control	98·9 ± 3·17	26·6 ± 1·2	112·9 ± 4·6	24·4 ± 0·78
2. Experimental	71·93 ± 2·31[a]	38·9 ± 2·3[a]	176·8 ± 7·6[a]	28·9 ± 0·93[a]
Cholesterol diet				
3. Control	128·8 ± 4·12	18·8 ± 1·6	88·8 ± 4·3	NS
4. Experimental	107·0 ± 3·42[a]	29·6 ± 1·8[a]	132·6 ± 6·4[a]	NS

*Values expressed as percentage increase in cholesterol in the serum in the Triton administered rats over the control rats given saline.
Values are the mean ± SEM for 12 rats. Group 2 has been compared with group 1 and group 4 with group 3. [a] $P < 0·01$.
NS, Not studied.

concentration of lipids in the serum may also be due to this decreased release of lipoproteins into the circulation.

Activity of lipoprotein lipase in the heart and adipose tissue and that of plasma LCAT

The activity of LPL in heart and adipose tissue showed significant increase in the rats given the material in both cholesterol free and cholesterol diet groups (table 4). This enzyme is involved in the uptake of circulating triglyceride rich lipoproteins (chylomicrons and VLDL) by the extra hepatic tissues. The increase in the activity of this enzyme may mean increased uptake of these lipoproteins and the decrease in the concentration of serum triglycerides may be due to this.

There was significant increase in the activity of plasma LCAT in the rats administered the material in the cholesterol free diet group. This enzyme is believed to be involved in the transport of cholesterol from the tissues to the liver for its catabolism and the decrease in the concentration of cholesterol in the tissues may agree with the increased activity of this enzyme in rats fed cholesterol free diet. The activity of this enzyme in rats given cholesterol diet was not studied.

There are a number of reports that pectin from various sources show hypocholesterolemic action (Bobek and Chrovathova, 1984; Frank *et al.,* 1986; Judd *et al.,* 1985; Kelly *et al.,* 1978). Most of these pectins have been reported to show activity only at a much higher level (Frank *et al.,* 1986) (500 mg and above/100 g body weight). The material now obtained from the juice of the stem of plantain, on the other hand, shows very significant lipid lowering effect at 20 mg/100 g body weight level. However this material may not be the only active substance present in the juice of plantain. This is because the juice has been found to be effective in clinical trials at a dose of about 500 ml/day. Either the juice may contain some other active principle also or it may reflect species difference, rat (a species normally resistant to atherosclerosis) requiring a higher dose.

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