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PREVENTION OF HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS IN RABBITS AFTER SUPPLEMENTATION OF *MYRISTICA FRAGRANS* SEED EXTRACT

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Abstract: *Myristica* seed extr. administration to hypercholesterolemic rabbits reduced serum cholesterol and LDL Cholesterol by 69.1% and 76.3% respectively and also lowered cholesterol/phospholipid ratio by 31.2% and elevated the decreased HDL-ratio significantly. *Myristica* seed extr. feeding also prevented the accumulation of cholesterol, phospholipids and triglycerides in liver, heart and aorta and dissolved atheromatous plaques of aorta by 70.9-76.5%. Fecal excretion of cholesterol and phospholipid were significantly increased in seed extract fed rabbits.

Key words : *Myristica seed* extr. atherosclerosis
fecal cholesterol HDL-ratio

INTROUDCTION

Hyperlipidemia is an important risk factor in the initiation and progression of the atherosclerotic lesions. The beneficial effect of lowering elevated serum cholesterol levels in the prevention of coronoary heart disease is well established (1).

Nutmeg the seeds of *M. fragrans* has been used for the treatment of heart ailments in Ayurvedic system of Medicine (2).

In Indian households it is used not only as an aromatic substance but also for flavour, spice and as a condiment (2). Preliminary studies from our laboratory showed cholesterol lowering activity of *M. fragrans* seed extract. The objective of this study was to examine the hypolipidemic effects of *M. fragrans* seed extract in cholesterol fed rabbits and to see whether *Myristica fragrans* seed extract feeding prevents aortic cholesterol accumulation.

METHODS

Authentic seeds of *M. fragrans* obtained from the National Institute of Ayurveda, Jaipur were powdered and defatted with petroleum ether (60-80°C). Defatted material was subjected to soxhlet extraction with ethanol (50% v/v) for 24 hr. Ethanol was removed under reduced pressure to obtain a brown solid. This extract was dissolved in 5 ml distilled water and administered orally by gastric intubation.

New Zealand white male rabbits weighing 1.5-2 kg were procured from the Central Drug Research Institute (CDRI), Lucknow. Rabbits were divided into five groups of six each. They were maintained on a standard pellet diet (Hindustan Lever Ltd.) plus fresh green leafy vegetables and water *ad libitum*. The average consumption of diet was 200 gm/day. Atherogenic diet was prepared by mixing wheat flour with milk powder, dried egg yolk, hydrogenated fat, butter, salt, jaggery and vitamin mixture in the given proportions.

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Contents	*Control (gm %)*	*Atherogenic (gm %)*
Protein	20	15
Carbohydrate	65	60
Sucrose 3	3	
Fat	5	15
Salts	4	4
Vitamin mix	1	1
Fiber	2	2

In addition to the atherogenic diet, each rabbit was given cholesterol powder 400 mg/kg b.wt. dissolved in 5 ml coconut oil.

Experimental design

Group I : Vehicle (5 ml distilled water) treated control (120 days).

Group II a and b : Atherogenic diet + 400 mg Chol/kg b.wt/day in 5 ml coconut oil for 60 days (Gr. IIa) and 120 days (Gr. IIb).

Group III : Atherogenic diet + cholesterol feeding for 60 days; from day 61-120, *Myristica* seed extr. (500 mg/kg b.wt/day) was given. Atherogenic diet and cholesterol feeding withdrawn from day 61-120 and kept on normal diet.

Group IV : Atherogenic diet + cholesterol feeding + *Myristica* seed extr. (500 mg/kg b.wt/day) from day 1-120 (concurrent feeding).

Blood was taken on day 0, 30, 60, 90 and 120 from the marginal ear vein and analysed for total Cholesterol (3), Triglyceride (4), Phospholipids (5), HDL-cholesterol (6), VLDL (7) and LDL Cholesterol (7). HDL-ratio was derived from the formula (HDL chol. x 100 to total cholesterol - HDL Chol.).

Animals were killed on day 61 and 121. Aorta were quickly removed, cleared of fat and connective tissue and examined for possible pathological changes in fresh as well as calcium formol fixed tissues. Lipid was extracted (8) from liver, heart muscles and aorta for cholesterol (3), triglyceride (4) and phospholipid (5) analysis.

Planimetric studies of ascending, thoracic and abdominal aorta were carried out with the help of camera lucida drawings for the quantitative estimation of aorta and plaque formation.

Fecal samples were collected from individual rabbits over a period of 7 days during the last month of feeding, collected feces were homogenized, extracted (chloroform : methanol), freeze dried and stored at —20°C. Fecal cholesterol (3) and phospholipid (5) were estimated.

RESULTS AND DISCUSSION

High fat diet in combination with cholesterol feeding raised the serum cholesterol, LDL and VLDL-cholesterol. At the end of 60 days treatment with *Myristica* ext. (gr. III), serum cholesterol, LDL-Chol. and VLDL Chol. were lowered by 69.1%, 76.3% and 56.6% respectively (Table I). This could be possibly due to an increase in the liver LDL-receptor activity (9, 10) and decreased hepatic triglyceride synthesis (11).

High cholesterol/phospholipid ratios are usually associated with atherosclerosis (12). The C/P ratio decreased significantly from1.74 to 1.13 after *Myristica* treatment. Concurrent treatment prevented the significant rise.

Serum HDL-ratio was increased in gr. III animals indicating beneficial effects of *Myristica* seed ext. feeding (Table I). Total cholesterol, phospholipid and triglyceride contents of liver, heart and aorta were high in atherofed groups ($P<0.001$. *M. fragrans* reduced tissue cholesterol to near normal levels. Similar reductions were also noticed in phospholipid and triglyceride contents of the tissues (Table II).

Planimetric studies showed that 23.0 - 36.8% of aortic wall was occupied by plaque in all the three regions (ascending, thoracic and abdominal) of the aorta in atherofed group. Regression (70.9-76.5%) was seen after *Myristica* feeding. Concurrent treatment did not induce plaque formation (12).

TABLE I : Effect of administration of *M. fragrans* seed extract on serum (mg/dl) and fecal (mg/gm) lipid profile of rabbits fed atherogenic diet. n=6 in each group, mean (± SEM).

Treatment	*Total cholesterol*	*Triglyceride*	*Phospholipid*	*HDL Chol.*	*VLDL Chol.*	*LDL Chol.*	*Chol./ Phosp. ratio*	*HDL ratio*	*Atherogenic index*	*Faecal Chol.*	*Faecal Phosp.*
I	110.3 ± 7.4	69.3 ± 4.5	159.2 ± 8.7	40.2 ± 3.7	13.9 ± 0.8	56.2 ± 3.6	0.69	57.34	1.74	56.3 ± 3.6	18.9 ± 0.6
IIa	632.9 ±** 18.3	220.8 ±** 29.8	372.3 ±** 22.6	153.12 ±** 8.3	44.2 ±** 5.9	434.6 ±** 20.1	1.60	32.52	3.12	72.2 ±* 0.8	21.9 ±* 0.4
IIb	1130.5 ±** 81.5	399.9 ±** 55.9	646.9 ±** 67.3	181.3 ±** 21.5	79.9 ±** 11.2	869.2 ±** 71.2	1.74	19.09	5.23	83.2 ±** 1.8	28.8 ±** 1.2
III	192.5± ** 20.9	95.8 ±** 14.1	172.5 ±** 11.4	64.1 ±* 8.9	19.2 ±** 2.8	102.9 ±** 14.6	1.10	49.86	1.90	126.1 ** + 4.0	42.5 ±** 0.7
IV	381.3 ±** 10.8	162.5 ±** 21.6	290.0 ±** 5.8	103.19 ±** 9.0	32.5 ±** 4.3	245.7 ±** 6.1	1.31	37.05	2.69	100.0 * + 3.6	34.5 ±* 1.1

*P<0.01; **P<0.001
Significance, Group IIa, IIb Vs Group I; Group III, IV Vs Group IIb.

TABLE II : Effect of *M. fragrans* extract treatment on cholesterol, triglyceride and phospholipids of liver, heart and aorta in athero fed rabbits.
n=6 in each group. Mean ± SE mg/g

	Cholesterol			*Triglyceride mg/gm*			*Phospholipid*		
	Liver	*Heart*	*Aorta*	*Liver*	*Heart*	*Aorta*	*Liver*	*Heart*	*Aorta*
I	7.5 ± 0.36	2.9 ± 0.08	4.1 ± 0.52	4.6 ± 0.24	4.2 ± 0.34	3.2 ± 0.37	7.4 ± 0.19	5.9 ± 0.39	5.3 ± 0.26
IIa	15.6 ±** 0.36	7.6 ±** 0.32	11.6 ±** 0.54	8.8 ±** 0.72	5.6 ±** 0.25	4.7 ±* 0.18	9.4 ±** 0.18	7.6 ±* 0.23	7.2 ±** 0.12
IIb	19.4 ±** 0.36	12.8 ±** 0.54	13.4 ±** 0.54	10.6 ±** 0.01	7.5 ±** 0.51	6.2 ±** 0.39	10.8 ±** 0.11	9.9 ±** 0.16	8.7 ±** 0.05
III	9.2 ±** 0.81	4.8 ±** 0.09	6.1 ±** 0.15	6.5 ±** 0.11	4.6 ±** 0.24	3.9 ±** 0.11	7.7 ±** 0.20	5.7 ±** 0.38	5.4 ±** 0.34
IV	10.9 ±** 0.54	5.3 ±** 0.17	9.0 ±** 0.12	7.8 ±** 0.17	5.0 ±* 0.22	4.0 ±** 2.12	8.4 ±** 0.12	6.5 ±** 0.05	6.2 ±** 0.10

*P<0.01; **P<0.001
Significance, Group IIa, IIb Vs Group I; Group III, IV Vs Group IIb.

The fecal cholesterol and phospholipid excretion was significantly increased in gr. III animals indicating beneficial effect of *Myristica* ext. feeding, (13).

In conclusion *Myristica fragrans* seed extract reduced experimentally induced atherosclerosis to a very large extent by decreasing the plaque size. Reductions in serum and tissue lipid parameters further highlight its hypolipidaemic activity, *M. fragrans* can therefore be considered as a potentially useful dietary supplement in the prevention of atherosclerosis in hyperlipidaemic patients.

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