

HYPOLIPIDAEMIC AND ANTIATHEROSCLEROTIC EFFECTS OF PLUMBAGIN IN RABBITS

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(Received on October 17, 1990)

Abstract : Plumbagin (2-methyl-5-hydroxy, 1:4 naphthoquinone) isolated from the roots of *Plumbago zeylanica* when administered to hyperlipidaemic rabbits, reduced serum cholesterol and LDL-Chol. by 53 to 86 percent and 61 to 91 percent respectively. It lowered cholesterol/phospholipid ratio by 45.8 percent and elevates the decreased HDL-Chol significantly. Further, Plumbagin treatment prevented the accumulation of cholesterol and triglycerides in liver and aorta and regressed atheromatous plaques of thoracic and abdominal aorta. Plumbagin treated hyperlipidaemic subjects excreted more fecal cholesterol and phospholipids.

In conclusion-Plumbagin feeding brings about a definite regression of atheroma and prevents the accumulation of cholesterol and triglycerides in liver and aorta.

Key words : Plumbagin HDL-Chol. Planimetry fecal excretion

INTRODUCTION

A causal relationship between blood cholesterol level and coronary heart disease is supported by an abundance of genetic, epidemiologic and experimental data. There is substantial evidence that lowering total, and particularly LDL cholesterol levels, will lead to reduction in the incidence of coronary heart disease which is still a leading cause of death. Because of putative linkage of serum cholesterol level to incidence of atherosclerosis, dietary manipulation of serum cholesterol has become a public health issue (1).

In a preliminary study, dietary feeding of Plumbagin was found to reduce serum cholesterol in cholesterol fed rats. The objective of this study is to further examine the hypolipidaemic effects of Plumbagin in cholesterol fed rabbits and to see whether Plumbagin prevents aortic cholesterol accumulation.

METHODS

Authentic plant material *Plumbago zeylanica*

was obtained from the National Institute of Ayurveda, Jaipur. Plumbagin was isolated from the roots according to the method of Roy and Dutta (2). Plumbagin (2-Methyl-5-hydroxy 1:4naphthoquinone) is a naphthoquinone derivative.

Adult healthy rabbits procured from Sheep and Wool Research Centre, Avikanagar, Tonk, Rajasthan were maintained at $26^{\circ} \pm 1^{\circ}C$ and were divided into five groups of six each. Group A served as placebo treated controls whereas groups B, C, D and E received atherogenic diet plus 400 mg/kg body weight cholesterol in 5 ml coconut oil for 120 days. Group D and E were subjected to Plumbagin treatment as detailed in experimental design.

EXPERIMENTAL DESIGN

- Group A: Vehicle treated control (120 days).
- Group B: Cholesterol feeding (400 mg/kg b. wt for 60 days).
- Group C: Chol. feeding (400 mg/kg b.wt for 120 days).

Group D: Chol. feeding (60 days) + Plumbagin (30 mg/kg. b.wt/day from day 61 to day 120).

Group E: Chol. feeding (60 days) + Plumbagin (30 mg/kg. b. wt/day from day 61 to day 180).

Atherogenic diet comprised of wheat flour base with addition of milk powder, dried egg, yolk, hydrogenated fat, salt, jaggery and vitamin mixture, and had the nutrient composition shown in Tables I, II-A and II-B.

The average consumption of diet was 200 g/day/rabbit.

Autopsies were performed at the termination of each treatment. Blood was collected through cardiac puncture, serum separated and stored at -20°C until assayed. Total cholesterol (3), triglyceride (4), phospholipid (5), HDL-Cholesterol (6) and LDL cholesterol (7) were estimated and statistically analysed using Student's 't' test.

Aorta and liver were quickly removed, cleared of adhering tissue and were prepared for histopathological examination as well as for biochemical analyses of cholesterol (3), phospholipid (5) and triglyceride (4). Planimetric studies of aortic dimensions were performed to study the progression and regression of intimal plaques.

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

RESULTS AND DISCUSSION

Plumbagin administration to rabbits fed the atherogenic diet resulted in the reduction of serum cholesterol and LDL cholesterol by 53.0 and 61.0% respectively (Table III-A; III-B). Marked decreased levels were noticed when plumbagin was given along with atherogenic diet (Group E Table III-A; III-B). Plumbagin treatment lowers VLDL-Chol. concentration (presumably β -VLDL) significantly in group D and E ($P \leq 0.001$, Table III-B). Higher cholesterol/phospholipid ratios are usually associated with atherosclerosis (8). The choles-

TABLE I : Composition of diet.

	Atherogenic %	Control %
Protein	14	14
Carbohydrate	56	61
Sugar	5	9
Fats	20	11
Salts	4	4
Vit. Mix	1	1

TABLE II-A : Ingredients of atherogenic diet.

Ingredients	Amount gms	Protein	Fats	Carbohydrate	Calorie K. Cal.	Cholesterol mg
Wheat flour	58	6.96	1.00	43.32	197.50	
Milk Powder	15	5.60	4.00	7.60	74.40	
Dried egg yolk	5	1.15	3.50	0.13	34.40	115
Jaggery	5	—	—	4.95	20.00	
Dalda fat	5	—	5.00	—	45.00	
Coconut oil	5	—	5.00	—	45.00	
Complex Vit. Mix	1	—	—	—	—	
Salt	4	—	—	—	—	
	100	13.71	19.62	55.98	416.30	115

TABLE II-B : Ingredients of control diet.

Ingredients	Amount gms	Protein	Fats	Carbohydrate	Calorie K. Cal.	Cholesterol mg
Wheat flour	65	7.86	1.10	45.12	221.17	
Milk Powder	15	5.60	4.00	7.60	74.40	
Dried egg yolk	—	—	—	—	—	—
Jaggery	9	—	—	8.90	36.00	
Dalda fat	3	—	3.00	—	27.00	
Coconut oil	3	—	3.00	—	27.00	
Complex Vit. Mix	1	—	—	—	—	—
Salt	4	—	—	—	—	—
	100	13.46	11.10	61.50	385.60	—

TABLE III-A: Serum Biochemistry of *Plumbagin* treated Rabbits.

Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	Phospholipid mg/dl	Total cholesterol Phospholipid ratio
A	95.2 ± 7.2	70.1 ± 3.2	140.4 ± 3.1	0.68 ± 0.23
B	625.0 ± 4.3 (+556.5)	228.4 ± 5.3 (+225.8)	278.6 ± 6.8 (+98.4)	2.24 ± 0.8 (+229.4)
C	1031 ± 32.4 (+982.9)	281.6 ± 6.5 (+301.7)	638.2 ± 6.9 (+354.6)	1.2 ± 0.1 (+76.5)
D	480 ± 2.3** (-53.4)	195.8 ± 6.5** (-11.6)	430.8 ± 7.2* (-32.5)	1.1 ± 0.3 (-8.3)
E	142.2 ± 25.6** (-86.2)	86.6 ± 12.1** (-69.2)	218.9 ± 3.0** (-65.7)	0.65 ± 0.1* (-45.8)

Figures in parentheses represent percent variation.

Group E & D vs C

*P ≤ .01

**P ≤ .001

TABLE III-B : Serum biochemistry of *Plumbagin* treated Rabbits.

Groups	HDL cholesterol mg/dl	HDL-Chol/ Total Chol.	VLDL-Chol. mg/dl Ratio	LDL-Chol mg/dl	HDL-Chol./ LDL-Chol. Ratio
A	36.0 ± 3.5	0.38	14.0 ± 1.8	45.2 ± 0.9	0.79
B	218.0 ± 6.4 (+ 505.5)	0.35	45.7 ± 1.1 (+ 226.4)	361.3 ± 3.2 (+ 699.3)	0.60
C	262.5 ± 7.2 (+ 629.2)	0.25	56.3 ± 1.3 (+ 302.1)	712.2 ± 19.9 (+ 1475.7)	0.37
D	162.0 ± 2.4** (- 38.3)	0.33*	39.2 ± 1.3** (- 30.4)	278.8 ± 1.4** (- 60.8)	0.58
E	56.9 ± 6.6** (- 78.3)	0.4*	17.3 ± 1.4** (- 69.3)	68.01 ± 3.7** (- 90.5)	0.83

Details as in Table III-A.

terol/phospholipid ratio decreased significantly from 1.2 to 0.65 (-45.8%) in concurrent fed rabbits (group E).

Epidemiologic studies have established the important role of HDL levels as a protective factor against coronary heart disease (9,10,11). HDL Chol/T. Chol ratio was increased from 0.25 to 0.4 ($P \leq 0.01$, Table III-B), indicating beneficial effects of Plumbagin treatment. Miller and Miller (12) have presented evidence that HDL is inversely related to total body cholesterol. Nestel and Miller

(13) postulated that HDL is involved in the transport of cholesterol from peripheral tissues into the liver. Glomset (14) showed that HDL alters the balance of nonesterified cholesterol between plasma and cells by increasing its utilization in the Lecithin/cholesterol acetyl Transferase (LCAT) system to form cholesterol ester which would move rapidly back into the cells.

Total cholesterol, triglyceride and phospholipid contents of liver and aorta were significantly high in the group fed the atherogenic diet. These levels

TABLE IV : Tissue biochemistry of *Plumbagin* treated Rabbits.

Groups	Cholesterol		Phospholipid		Triglyceride	
	Liver	Aorta	Liver	Aorta	Liver	Aorta
	← mg/g →		← mg/gm →		← mg/g →	
A	9.25 ± 0.2	3.9 ± 0.4	4.5 ± 0.5	4.02 ± 0.4	3.1 ± 0.04	2.25 ± 0.12
B	14.6 ± 1.01	10.8 ± 0.3	14.15 ± 0.4	11.5 ± 1.3	4.5 ± 2.4	4.5 ± 0.4
C	33.75 ± 0.2	18.4 ± 0.15	15.9 ± 0.3	13.8 ± 0.2	9.9 ± 1.6	8.3 ± 0.8
D	13.2 ± 0.2**	9.6 ± 1.5**	12.1 ± 0.2**	8.2 ± 0.6**	4.5 ± 1.1**	3.9 ± 0.2**
E	7.9 ± 0.6**	6.6 ± 1.03**	5.6 ± 0.4**	4.3 ± 0.4**	3.2 ± 0.2**	2.45 ± 0.05**

Comparisons as in Table III-A

TABLE V : Rabbit-Fecal Biochemistry.

Groups	Serum Cholesterol mg/dl	Cholesterol in excreta mg/gm	Serum Phospholipid mg/dl	Phospholipid in excreta mg/gm
A	95.2 ± 7.2	40.6 ± 1.3	140.4 ± 3.1	19.47 ± 0.6
B	625.0 ± 4.3	74.2 ± 3.8	278.6 ± 6.8	26.6 ± 2.1
C	1031 ± 32.4	105.0 ± 3.2	638.2 ± 6.9	70 ± 1.1
D	480 ± 2.3**	107.5 ± 4.3	430.8 ± 7.2**	41.3 ± 2.6**
E	142.2 ± 25.6**	53.4 ± 1.7**	218.9 ± 3.0**	32.2 ± 0.9**

Comparisons were made as given in Table III-A.

TABLE VI : Planimetric studies of Thoracic and Abdominal Aorta.

Groups	Aortic Lumen %		Plaque%		Total wall Area%	
	Thoracic	Abdominal	Thoracic	Abdominal	Thoracic	Abdominal
A	49.25 ± 1.6	48.5 ± 2.3	—	—	50.74 ± 1.6	51.5 ± 2.3
B	14.2 ± 1.63	12.97 ± 0.7	36.2 ± 6.5	30.1 ± 5.6	85.8 ± 1.63	87.02 ± 0.73
C	14.5 ± 2.1	11.6 ± 1.4	49.1 ± 2.5	32.13 ± 4.9	85.47 ± 1.0	88.4 ± 2.8
D	22.4 ± 0.4*	21.4 ± 3.04	23.6 ± 3.3**	10.03 ± 1.1**	77.7 ± 0.5**	78.75 ± 3.1
E	28.3 ± 3.7*	41.36 ± 2.3**	—	—	70.75 ± 1.4**	58.64 ± 4.2**

Comparisons as in Table III-A.

could be brought to normal when treated with plumbagin (Table IV).

Plumbagin fed rabbits excreted more fecal cholesterol and phospholipids (Table V) suggesting that modulation of absorption was affected. Dietary fats modulate and metabolize chylomicrons to chylomicron remnants for ultimate removal by the liver (15)

Atherogenic diet produced atheromatous lesions in the thoracic and abdominal portions of the aorta. These lesions were highly proliferative

with foam cell formation. Planimetry studies showed 49.1 percent of aortic wall was occupied by plaque in athero fed group while 23.6 percent area was occupied by plaque after plumbagin feeding (Table VI). Possible mechanism for prevention of plaque formation may be that HDL inhibits the uptake of LDL by arterial wall and also facilitates the transport of cholesterol from peripheral tissues to the liver where it is catabolised and excreted out of the body (16). Prevention of plaque formation in atheromatous rabbits encourages us to undertake these studies in human subjects with established atheroma.

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