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Prevention of aortic lesions and hyperlipidaemia by alfalfa seed extract in cholesterol fed rabbit

V. P. DIXIT, S. C. JOSHI and PRABHA JAIN
Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur 302 004, India

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Abstract. Extract of alfalfa seed (ethanolic 50 % v/v) prevents the development of plaque formation and hyperlipidaemia in cholesterol fed rabbits. It inhibits the elevation of serum total cholesterol, triglycerides, phospholipids, LDL-cholesterol and total cholesterol/phospholipid ratio, while HDL-cholesterol/total cholesterol ratio increases, which is associated with a reduced incidence of atherosclerosis. Further reduction in total cholesterol and phospholipid contents of liver and heart muscle are suggestive of a beneficial role of the seed extract. The possible mechanisms of action are discussed.

Keywords. Alfalfa seed extract (ethanolic 50% v/v); HDL-cholesterol; atherosclerosis; hyperlipidaemia; LDL-cholesterol.

Introduction

The relationship between lipid and atherosclerosis has been a subject of extensive investigation over the last 6 decades. Cholesterol added to diet (0·5-2 %) results in hypercholesterolemia (Shore *et al.,* 1974; Wissler and Verselinovitch, 1974) and subsequently in the induction and development of atherosclerosis (Brown *et al.,* 1965).

Alfalfa meals prevent hypercholesterolemia in rabbits (Yanaura and Sakamoto, 1975) and cynomologus monkeys (Malinow *et al.,* 1978). Antiatherosclerotic activity of alfalfa seed extract (ethanolic 50 % v/v) in chicks has been reported from our laboratory (Dixit and Joshi, 1985).

The aim of the present study was to examine whether alfalfa seed extract feeding prevents of aortic lesion and hypercholesterolemia in cholesterol fed rabbits.

Materials and methods

Medicago sativa (alfalfa) seeds obtained from the Seed Corporation of India were powdered and defatted with petroleum ether (60-80°). Defatted material was subjected to Soxhlet extraction with Ethanol (50 % v/v) for 24 h. Ethanol was removed under reduced pressure to obtain a brown solid.

Eighteen adult healthy rabbits were used. They were maintained in an airconditioned room (26° ± 1°C) and were divided into groups of 6 each. Group A served as controls. Group B received 500 mg cholesterol in 5 ml coconut oil/day for a period of 4 months.

Group C received 500 mg cholesterol in 5 ml coconut oil/day + 500 mg of ethanolic (50 %) extract of alfalfa seeds/day.

All animals were killed after a 4 month period. The blood was collected through cardiac puncture, serum separated and stored at — 20°C until assayed. Total cholesterol (Zlatkis *et al.,* 1953), triglyceride (Gottfried and Rosenberg, 1973), phospholipid (Zilversmit and Davis, 1950), HDL-cholesterol (Burnstein *et al.,* 1976) and LDL-cholesterol (Dedonder-Decoopman *et al.,* 1980) were estimated and statistically analysed using Student's *'t'* test.

Aorta, liver and heart muscles were quickly removed, cleared of fat and connective tissue. Aortas were prepared for histopathological examinations. The liver and left ventricular heart muscles were frozen and analysed for glycogen (Montgommery, 1957), cholesterol (Zlatkis *et al.,* 1953), phospholipid (Zilversmit and Davis, 1950) and triglyceride (Gottfried and Rosenberg, 1973).

Toxicity Studies

Male rats of inbred colony (body wt. 170 ± 13 g) were assigned randomly to 3 groups of 8 animals each. These groups were maintained on rat feed pellets (Hindustan Lever Ltd.). After first 4 days, 1 % cholesterol was added to the diets of group 2 and 3 animals. Group 1 served as control, while groups 2 and 3 were administered orally 100 mg and 300 mg/day of the ethanolic extract of alfalfa seed for 2-6 months. All animals of group 3 and half each from groups 1 and 2 were killed after 2 months, while the remaining were killed after 6 months.

No deaths occurred during the experiments and seed extract had no inhibitory effect on body weight. Serum cholesterol and triglycerides were found to be low. At post-mortem, no gross pathological changes were observed.

Results

Biochemical changes

The body and liver weights were reduced significantly in rabbits fed with cholesterol + alfalfa simultaneously (Group C) when compared with Group B (table 1). The results presented in table 2 indicate that the cholesterol contents of liver and heart muscles of rabbits fed with cholesterol + alfalfa seed extract were significantly reduced. No significant change in triglyceride contents of liver was noticed, but a significant reduction was noticed in that of the heart muscle. Phospholipid contents of liver and heart muscles were reduced significantly. Glycogen contents of liver and heart muscles were also reduced. A significant reduction in serum total cholesterol, phospholipid, triglyceride, LDL-cholesterol, VLDL-cholesterol and total cholesterol/phospholipid ratio was observed in rabbits fed with cholesterol + alfalfa (Group C) compared to cholesterol feeding alone (Group B), while HDL-cholesterol/total cholesterol ratio was raised after combined feeding (table 3).

Histopathological changes in aorta

Cholesterol fed rabbits showed lesions in aortic arch characterised by a thickened intima, cell proliferation, collagen and abundant lipid accumulation (figure 1).

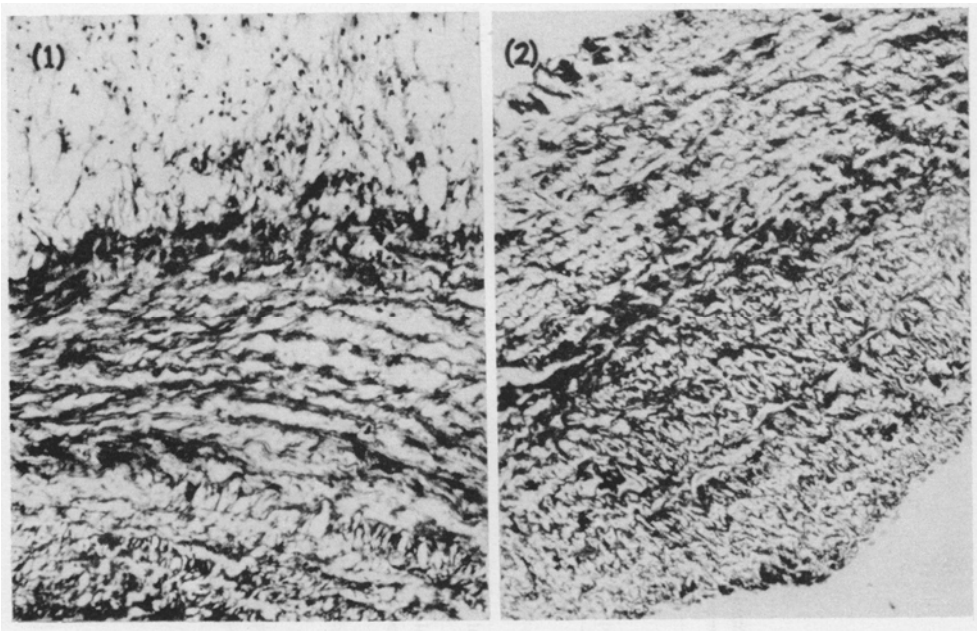
Table 1. Changes in the body, liver and heart weight after cholesterol/alfalfa seed extract feeding.

Treatment	Body weight (kg)		Liver (mg/100 gm body weight)	Heart
	Initial	Final		
Group A (control)	1·68 ± 0·09	1·62 ± 0.08	2603 ± 26.15	203·0 ± 7·0
Group B (cholesterol feeding)	1·66 ± 0·1	1·47 ± 0·06	4216·0 ± 64·0	213·60 ± 4·37
Group C (cholesterol + alfalfa feeding)	1·43 ± 0·1	1·08 ± 0·11[c]	2661·9 ± 40·2[b]	198·69 ± 7·6[a]

[a] *P*: Not significant when Group C is compared with Group B.
[b] $P \leqslant 0{\cdot}01$ when Group C is compared with Group B.
[c] $P \leqslant 0{\cdot}001$ when Group C is compared with Group B.

The values represent Mean ± SEM.

Calcification of tunica media was conspicuous. Such alterations did not occur in the aorta of rabbits which were maintained on simultaneous feeding with cholesterol and alfalfa seed extract (figure 2).



Figures 1 and 2. 1. Aorta of cholesterol fed rabbit showing cell proliferation, collagen and lipid accumulation. × 100 HE. **2.** Aorta ofrabbit fed with cholesterol and alfalfa seed extract. Note the normal architecture of the aortic wall. × 100 HE.

Table 2. Changes in tissue lipids after cholesterol/alfalfa seed extract feeding.

Treatment	Cholesterol (mg/gm)		Triglyceride (mg/gm)		Phospholipid (mg/gm)		Glycogen (mg/gm)	
	Liver	Heart muscle	Liver	Heart muscle	Liver	Heart muscle	Liver	Heart muscle
Group A (control)	10·93 ± 0·15	9·12 ± 0·07	3·96 ± 0·05	4·37 ± 0·18	8·32 ± 0·08	10·02 ± 0.08	5·71 ± 0·04	1·10 ± 0·09
Group B (cholesterol feeding)	15·5 ± 0·3	17·25 ± 0·25	5·50 ± 0·20	6·0 ± 0·2	13·0 ± 0·10	11·5 ± 0·20	5·73 ± 0·14	2·85 ± 0·15
Group C (cholesterol + alfalfa feeding)	11·34 ± 0·06[c]	9·5 ± 0·03[c]	5·34 ± 0·06[a]	5·02 ± 0·03[b]	9·25 ± 0·07[c]	10·71 ± 0·06[b]	5·09 ± 0·005[b]	1·23 ± 0·007[c]

[a] *P*: Not significant when Group C is compared with Group B.
[b] $P \leqslant 0{\cdot}01$.
[c] $P \leqslant 0{\cdot}001$.
The values represent Mean ± SEM.

Table 3. Serum analysis of cholesterol/alfalfa seed extract fed rabbits.

Treatment	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Phospholipid (mg/dl)	HDL-cholesterol/ total cholesterol ratio	Total cholesterol/ phospholipid ratio
Group A (control)	114·6 ± 8·8	49·66 ± 3·28	58·18 ± 4·36	12·31 ± 0·99	61·58 ± 3·6	126·6 ± 3·42	0·433 ± 0·04	0·903 ± 0·25
Group B (cholesterol feeding)	768 ± 8·5	122·88 ± 0·255	179·1 ± 4·16	35·83 ± 1·68	609·17 ± 10·16	267·6 ± 5·32	0·16 ± 0·03	2·869 ± 0·15
Group C (cholesterol + alfalfa feeding)	156 ± 29·0[a]	74·88 ± 1·682[a]	76·33 ± 24·36[a]	15·26 ± 4·87[a]	71·73 ± 41·65[a]	135·1 ± 20·06[a]	0·48 ± 0·058[a]	1·154 ± 0·145[a]

[a] $P \leqslant 0{\cdot}001$ when Group C is compared with Group B.
The values represent Mean ± SEM.

Discussion

The ethanolic extract of *Medicago sativa* seeds contain two main aglycones namely, medicagenic acid and hederagenin (Malinow, 1984). Alfalfa seed extract prevents the development of plaque formation and hyperlipidaemia. The possible mechanism for prevention of plaque formation may be that HDL-inhibits the LDL-arterial wall uptake and also facilitate the transport of cholesterol from peripheral tissue to the liver, where it is catabolised and excreted out of the body (Carew *et al.,* 1976).

Reduction in hyperlipidaemia occurs simultaneously with an increase in the HDL-cholesterol/total cholesterol ratio which is associated with a reduced incidence of atherosclerosis (Castelli *et al.,* 1977). Decrease in total cholesterol-phospholipid ratio after alfalfa feeding indicates the antiatherogenic nature of the plant product. Further reduction in total cholesterol and phospholipid contents of liver and heart muscle may be suggestive of a beneficial role for the drug.

Malinow *et al.* (1980a, b) suggested that alfalfa ingestion decreased the intestinal absorption and exogenous and endogenous cholesterol and increased the bile acid excretion. Jackson (1981) demonstrated that alfalfa meal contain high levels of an immunoreactive thyrotropin releasing hormone like material (IR-TRH), a finding that suggests another possible mechanism of action.

Finally it seems that the ethanolic extract (50 % v/v) of alfalfa seed is beneficial in reducing hyperlipidaemia and prevents atherosclerosis. The apparent lack of toxicity advocates its long term use. However, long term tolerance studies are needed before being recommended for human use.

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