

ANTIATHEROSCLEROTIC EFFECTS OF ALFALFA MEAL INGESTION IN CHICKS : A BIOCHEMICAL EVALUATION

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Summary : Feeding Alfalfa seed extract to chicks resulted in significant reduction of total cholesterol, phospholipid, triglyceride, LDL-Cholesterol and VLDL-cholesterol. Simultaneously, an increase in the HDL-cholesterol/total cholesterol ratio occurred with reduction in total cholesterol and phospholipid contents of liver and (ventricular) muscle of the heart. Results obtained with alfalfa seed extract ingestion were evaluated with a standard drug, compound, clofibrate.

Key words : hyperlipidaemia
alfalfa seed extract

antiatherosclerosis
HDL-cholesterol

INTRODUCTION

Alfalfa meals prevent hypercholesterolemia, triglyceridemia and atherogenesis in cholesterol fed rabbits (4, 13) and *Cynomolgus* monkeys (9). Alfalfa saponins decreased intestinal absorption of fats (8) and prevent the expected increase in plasma cholesterolemia induced by dietary cholesterol (8,10,11).

Present studies were designed to test this premise in chicks and to compare such results with clofibrate.

MATERIAL AND METHODS

Forty healthy chicks (30 day old, 300-350 g) were kept on standard diet (wheat bran 7 kg, rice 12 kg, sesame "Til" 5 kg, fish meal 5 kg, groundnut cake 5 kg, molasses 3 kg, bone meal, 1 kg, lime stone 1 kg and salt 0.25 g).

They were placed in pairs inside the two tier cages (18"x16"x14") and were provided optimum condition of ventilation, light, temperature and sanitation. Chicks

were divided into groups of 10 each : Group A received standard diet + 200 mg cholesterol. Group B received standard diet + 200 mg cholesterol + 200 mg alfalfa seed extract (50% ethanolic extract of alfalfa seeds) : Group C was kept on standard diet + 200 mg cholesterol + 200 mg clofibrate. The remaining 10 chicks were kept on standard diet and served as normal controls. The drugs were administered orally each day for 3 weeks. Chicks were sacrificed 24 hr after the administration of the last dose. Blood was withdrawn through cardiac puncture and serum analysed for total cholesterol (15), phospholipid (14), triglyceride (6), HDL-cholesterol (1), VLDL-cholesterol and LDL-cholesterol (5).

The cardiac muscle from the left ventricle and liver pieces were frozen and analysed for glycogen (12), cholesterol (15), triglyceride (6) and phospholipid (15).

RESULTS

Serum analysis – A significant reduction in serum total cholesterol, phospholipid, triglyceride, VLDL-cholesterol, LDL-cholesterol and total cholesterol phospholipid ratio were noticed in chicks fed with alfalfa seed extract (Gr. B) or clofibrate (Gr. C) in comparison with cholesterol feeding alone (Gr. A). while a significant increase in HDL-Chol./total cholesterol ratio was recorded in chicks treated with alfalfa and clofibrate separately (Table I).

TABLE I : Changes in the various lipid fractions in chicks fed with atherogenic diet and treated with alfalfa seed extract or clofibrate.

Treatment	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)	VLDL cholesterol (mg/dl)	LDL-cholesterol	HDL chol./total chol. ratio	Cholesterol/Phospholipid ratio
Non-treated control	150.0 ±7.0	510.0 ±6.0	130.0 ±5.0	10.0 ±1.0	100.0 ±5.0	0.266 ±0.02	1.15 ±0.14
A - Cholesterol feeding	1240.0 ±110.0	249.9 ±30.0	235.2 ±20.0	49.98 ±6.0	1016.0 ±90.42	0.140 ±0.01	5.27 ±0.43
B - Cholesterol alfalfa	566.0 ^b ±37.0	50.7 ^a ±10.0	168.8 ^a ±17.0	10.14 ^c ±2.0	437.4 ^a ±50.0	0.209 ^b ±0.01	3.53 ^a ±0.25
C - Cholesterol + Clofibrate	572.0 ^b ±60.0	42.1 ^a ±7.5	166.5 ^a ±15.0	8.42 ^c ±3.0	467.58 ^a ±55.0	0.167 ^a ±0.003	3.43 ^a ±0.27

Value differs significantly from 'A' (a - $P < 0.05$; b - $P < 0.01$; c - $P < 0.001$)

TABLE II : Changes in the tissue lipids and glycogen in chicks fed with atherogenic diet and treated with alfalfa seed extract or clofibrate.

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
Non-treated Control	6.8±0.7	2.3±0.4	2.95±0.5	3.2±0.2	7.1±0.4	5.7±0.5	15.0±2.0	2.9±0.8
A - Cholesterol feeding	11.0±1.0	4.37±0.3	3.91±0.7	6.21±0.2	12.4±0.7	10.1±1.0	54.2±3.5	8.7±1.0
B - Cholesterol + alfalfa	6.72±0.8 ^a	2.95±0.5 ^b	3.95±0.8 ^d	4.78±0.4 ^a	8.79±0.9 ^a	6.5±0.7 ^a	43.1±2.1 ^a	4.34±0.9 ^a
C - Cholesterol + clofibrate	4.87±0.9 ^b	3.32±0.1 ^a	3.96±0.5 ^d	4.80±0.3 ^a	8.84±0.9 ^a	6.1±0.7 ^a	39.0±3.5 ^a	4.52±0.7 ^a

Value differs when compared with 'A' (a P<0.05; P<0.01; d - P : non-significant).

Tissue biochemistry (Table II) :

Cholesterol – Cholesterol contents of liver were reduced in chicks treated with alfalfa and clofibrate. Similar reduction was also noticed in the ventricular muscles of chicks fed with cholesterol + alfalfa or clofibrate.

Triglyceride – No significant change in TG contents of liver was noticed in chicks treated with alfalfa or clofibrate, but a significant reduction was noticed in ventricular muscle TG contents in chicks treated with alfalfa or clofibrate (Table II).

Phospholipid – Phospholipid contents of liver and heart muscle were significantly reduced in both groups of chicks treated with alfalfa or clofibrate (Table II).

Glycogen – Glycogen contents of liver and ventricular muscle of the heart were significantly reduced in chicks treated with alfalfa or clofibrate.

DISCUSSION

The VLDL – cholesterol is known to be an atherogenic factor (7). Reduction in hyperlipidaemia occurs simultaneously with an increase of the HDL-cholesterol/total cholesterol ratio – which is associated with a reduced incidence of atherosclerosis (2).

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 ventricular muscles of the heart may be suggestive of beneficial role of the drug.

Alfalfa ingestion decreased the intestinal absorption of exogenous and endogenous and increases the bile acid excretion (10). It was further suggested that these effects were attributed to the saponin contents of the seed diet (11). Alfalfa is probably one of the plant material so far known which is effective in both hyperlipidaemic and atherosclerosis, since it lowers the cholesterol, TG, phospholipids, LDL-cholesterol and VLDL-cholesterol ratio which has been shown to an independent "antiatherosclerotic factor" (2). Finally, it seems that alfalfa seed extract is effective like clofibrate in reducing hyperlipidaemia.

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