

## HYPOLIPIDAEMIC EFFECTS OF MEDICAGO SATIVA SEED EXTRACTS (50% EtOH) IN RABBITS UNDER EXPERIMENTAL CONDITIONS

V.P. DIXIT & PRABHA JAIN

*Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur – 302 004, India.*

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**ABSTRACT:** Increased serum cholesterol and LDL cholesterol were reduced by 38 – 41.7% and 48 – 53.3% respectively when fed with alfalfa seed extract from the beginning or in established hyperlipidaemic model. LDL-cholesterol lowering was maximum (64.4%) in a model fed with alfalfa meals without cholesterol. An increase in HDL- cholesterol total cholesterol is suggestive of beneficial role since it is associated with low incidence of atherosclerosis. Possible mechanism of lipid lowering activity of *Medicago sativa* seed extract is worked out.

### INTRODUCTION

Alfalfa meals prevent hypercholesterolemia, triglyceridemia and atherogenesis in cholesterol fed rabbits (1,2) and cynomolgus monkeys (3). Alfalfa saponins reduced intestinal absorption of cholesterol in rats (4) and prevent cholesterolemia in cholesterol fed monkeys (5). The present studies were designed to test this premise in hyperlipidaemic rabbits under different experimental conditions.

### MATERIALS AND METHODS

Thirty adult healthy rabbits of both sexes were used. They were maintained in an air-conditioned room ( $26^{\circ} \pm 1^{\circ}\text{C}$ ) and were provided with rabbit feed. The rabbits were divided into groups of 6 each. Gr. A – animals served as controls. Gr. B – animals received cholesterol (500 mg | day in 5 ml. coconut oil) for a period of 45 days and then divided into two separate groups named as B<sub>1</sub> and B<sub>2</sub>. Animals of Gr. B<sub>1</sub> continued to receive cholesterol till 90 days while the

animals of Gr. B<sub>2</sub> received cholesterol (500 mg | day in 5 ml. coconut oil) + 50% ethanolic (v/v) alfalfa seed extract till 90 days. Gr. C – animals received cholesterol + alfalfa seed extract for a period of 45 days and then divided into two separate groups viz., Gr. C<sub>1</sub> and Gr. C<sub>2</sub>. animals of Gr. C<sub>1</sub> continued to receive cholesterol + alfalfa seed extract till 90 days. Whereas in Gr. C<sub>2</sub> animals – cholesterol was stopped at 45 days but alfalfa seed extract administration continued till 90 days. Blood samples were taken on day 45 and 90. Serum was separated and analysed for total cholesterol (6), phospholipids (7), triglyceride (8), nonestrified free fatty acid (9), VLDL – cholesterol, LDL – cholesterol (10) and HDL – cholesterol (11).

### RESULTS AND DISCUSSION

A significant increase in the serum total cholesterol, triglyceride, phospholipids, NEFA, LDL-cholesterol, and VLDL-

cholesterol was observed after cholesterol feeding for a period of 45 days and 90 days (Gr.B<sub>1</sub> Table – 1). The HDL Chol.| Total cholesterol ratio was reduced after 90 days of cholesterol feeding (Table – 1); Serum lipid levels were kept low by simultaneous feeding with alfalfa seed extract (Gr. C<sub>1</sub>). These levels lowered further when cholesterol feeding was withdrawn (Gr. C<sub>2</sub>).

Alfalfa seed extract feeding induced a significant reduction in various functions of lipid and lipoprotein cholesterol while HDL

– Chol. | total cholesterol ratio increased significantly which is associated with reduced incidence of atherosclerosis (2).

Alfalfa meal decreased the intestinal absorption of endogenous cholesterol and increased the bile acid excretion. These effects were attributed to the saponin content of the seed (13, 14). Alfalfa meal contains high levels of an immuno – reactive thyrotropin releasing hormone like material (IR – TRH), a finding suggests another possible mechanism (15).

**TABLE – 1**

**Changes in the serum lipid levels after alfalfa seed ext. (50% EtOH) Treatment in cholesterol fed rabbits**

| Treatment  | Total Cholesterol     |                        | Triglyceride              |                           | Phospholipid             |                           | Non-Esterified Free Fatty Acid (mEg/L) |                            | VLDL-Cholesterol (mg/dl) |                         | LDL-Cholesterol (mg/dl)   |                           | HDL Cholesterol Total Cholesterol Ratio |                            | HDL-Cholesterol (mg/dl)  |                          |
|--|-----------------------|------------------------|---------------------------|---------------------------|--------------------------|---------------------------|--|----------------------------|--------------------------|-------------------------|---------------------------|---------------------------|---|----------------------------|--------------------------|--------------------------|
|  | 45 days               | 90 days                | 45 days                   | 90 days                   | 45 days                  | 90 days                   | 45 days                                | 90 days                    | 45 days                  | 90 days                 | 45 days                   | 90 days                   | 45 days                                 | 90 days                    | 45 days                  | 90 days                  |
| Control (Gr.A)   | 114.0 ± 8.7           | 110.0 ± 5.0            | 58.2 ± 4.4                | 48.2 ± 4.0                | 126.7 ± 3.4              | 106.7 ± 3.0               | 0.235 ± 0.007                          | 0.205 ± 0.002              | 12.4 ± .99               | 10.4 ± 1.0              | 61.58 ± 3.6               | 69.0 ± 7.6                | 0.433 ± .04                             | 0.483 ± .02                | 49.6 ± .02               | 53.1 ± 3.5               |
| Cholesterol Feeding for 90 days (Gr. B <sub>1</sub> )  | 657 ± 37              | 1160 ± 40 <sup>d</sup> | 205.6 ± 12.0 <sup>d</sup> | 278.7 ± 12.8 <sup>d</sup> | 107.1 ± 2.7 <sup>d</sup> | 236.8 ± 35.3 <sup>d</sup> | 0.342 ± 0.002 <sup>d</sup>             | 0.355 ± 0.01 <sup>d</sup>  | 41.1 ± 2.8 <sup>d</sup>  | 53.7 ± 2.1 <sup>c</sup> | 429.8 ± 52.5 <sup>c</sup> | 874.3 ± 29.9 <sup>d</sup> | 0.252 ± 0.10 <sup>a</sup>               | 0.20 ± 0.1 <sup>d</sup>    | 165.6 ± 10.5             | 232.0 ± 28.9             |
| Deviation  | 10.5 fold ↑           |                        | 15.8 fold ↑               |                           | 2.2 fold ↑               |                           | 1.7 fold ↑                             |                            | 5.2 fold ↑               |                         | 12.7 fold ↑               |                           | 58.6                                    |                            |                          |                          |
| Cholesterol Feeding 45 days, then Cholesterol + alfalfa for next 45 days. Total duration 90 days (Gr. B <sub>2</sub> ) | 600 ± 80              | 372 ± 164              | 222.5 ± 22.5              | 190.0 ± 10.0 <sup>c</sup> | 194.1 ± 2.9              | 173.3 ± 5.3 <sup>a</sup>  | 0.342 ± 0.002                          | 0.322 ± 0.05 <sup>b</sup>  | 42.5 ± 2.5               | 38.0 ± 2.0 <sup>c</sup> | 392.5 ± 62.5              | 204.0 ± 12.0 <sup>d</sup> | 0.276 ± .12 <sup>b</sup>                | 0.349 ± 0.1 <sup>d</sup>   | 165.6 ± 11.7             | 129.8 <sup>d</sup> ± 8.7 |
| Deviation  | -38%                  |                        | -14.6%                    |                           | -10.7%                   |                           | -5.8%                                  |                            | -10.6%                   |                         | -48.1%                    |                           | +1.26 fold ↑                            |                            |                          |                          |
| Cholesterol + Alfalfa for 90 days (Gr. C <sub>1</sub> )  | 470 ± 10 <sup>d</sup> | 274 ± 14 <sup>d</sup>  | 191.7 ± 0.6 <sup>a</sup>  | 127.1 ± 2.8 <sup>d</sup>  | 182.4 ± 2.9 <sup>b</sup> | 171.3 ± 3.7 <sup>a</sup>  | 0.343 ± 0.001 <sup>a</sup>             | 0.333 ± 0.003 <sup>a</sup> | 38.3 ± 0.50 <sup>a</sup> | 25.4 ± .4 <sup>d</sup>  | 312.7 ± 29.0 <sup>a</sup> | 139.7 ± 8.0 <sup>d</sup>  | 0.254 ± 0.006 <sup>a</sup>              | 0.399 ± 0.001 <sup>d</sup> | 119.4 ± 5.6 <sup>a</sup> | 109.3 ± 8.5 <sup>d</sup> |
| Deviation  | -21.7%                | -41.7%                 | -33.7%                    |                           | -6.1%                    |                           | -2.9%                                  |                            | 33.7%                    |                         | 55.3%                     |                           | 1.57fold                                |                            |                          |                          |
| Cholesterol +  | 337                   | 156.0                  | 135.4                     | 76.3 ±                    | 174.2                    | 135.2                     | 0.291 ±                                | 0.273 ±                    | 27.1                     | 15.3                    | 201.2                     | 71.7 ±                    | 0.320 ±                                 | 0.480 ±                    | 108.0 ±                  | 74.9                     |

|   |              |              |             |          |             |              |           |           |             |             |              |          |           |           |         |             |
|---|--------------|--------------|-------------|----------|-------------|--------------|-----------|-----------|-------------|-------------|--------------|----------|-----------|-----------|---------|-------------|
| Alfalfa upto 45 days  | $\pm 10.3^d$ | $\pm 29.9^b$ | $\pm 1.5^d$ | $24.4^d$ | $\pm 1.3^b$ | $\pm 20.1^a$ | $0.055^a$ | $0.025^a$ | $\pm 0.7^d$ | $\pm 4.1^a$ | $\pm 10.1^d$ | $41.7^a$ | $0.008^d$ | $0.058^d$ | $3.8^d$ | $\pm 6.1^d$ |
| Then cholesterol feeding stopped and Alfalfa continued till 90 days (Gr.C2) |              |              |             |          |             |              |           |           |             |             |              |          |           |           |         |             |
| Deviation   | 53.7%        |              | 43.7%       |          | 22.4%       |              | -6.2%     |           | 43.5%       |             | 64.4%        |          | 1.5 fold↑ |           |         |             |

a =  $P \leq$  Non significant ; b =  $P \leq .01$ ; d =  $P \leq 0.001$

Gr. B<sub>1</sub> Compared with Gr. A

Gr. B<sub>2</sub> Compared with Gr. B<sub>1</sub>

Gr. B<sub>1</sub> Compared with Gr. C<sub>1</sub>

Gr. C<sub>2</sub> Compared with Gr. C<sub>1</sub>

All figures  $\pm$  SEM.

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