

Hypocholesterolemic effect of *Terminalia chebula* fruit (Myrobalan) in mice

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ABSTRACT : Cholesterol fed mice were administered powdered myrobalan, the fruit of *Terminalia chebula*, to evaluate its antiatherogenic activity. Male mice were fed a diet containing 1% cholesterol with or without myrobalan for 100 days. The cholesterol containing diet fed to mice caused increased food intake, body weight, serum cholesterol, triglyceride, thickening of the walls of aorta and shrinkage in its lumen (group 2). The oral administration of myrobalan to mice on atherogenic diet successfully reversed these effects (group 3). However, the food intake was observed to be high as compared to the control animals. Control animals (group 1) received only the vehicle. The results suggest that myrobalan has hypocholesterolemic effect in animals fed with atherogenic diet.

Key words : Myrobalan, hypocholesterolemia, cholesterol, triglyceride

INTRODUCTION

Myrobalan, the fruit of *Terminalia chebula* (Combretaceae) was administered orally to mice in order to assess its hypocholesterolemic action. It has been reported that myrobalan has a curative role in heart diseases and in obesity in humans¹⁻². As direct relationship exists between the serum cholesterol and cardiovascular diseases³, it is of interest to find medicinal plants and their products which may be used in lowering serum cholesterol⁴. However, experimental evidence for the hypocholesterolemic action of myrobalan is limited. Therefore, in the present study, the hypocholesterolemic action of myrobalan was evaluated in cholesterol fed mice.

EXPERIMENTAL DESIGN

Six months old male swiss albino isogenic mice were obtained from The Biological Production Division, Government Veterinary College, MHOW (M.P.) Mice were housed in polypropylene cages with free access to drinking water and food. The feed consisted of 36.50% maize, 36.50%

jowar, 18.50% wheat, 7.50% gram and 1% NaCl.

The fruits of myrobalan were purchased from local market and were gently baked and ground to fine powder. The aqueous suspension of powdered myrobalan was prepared and 0.1ml was administered orally to mice at a calculated dose of 128 mg/kg body weight/day (3.2mg/25gm mouse/day). Human dose is 3-9gm/day for an adult of 70kg body weight, therefore dose ranges from 42.85mg/kg to 128.57mg/kg. This is the basis of selecting the present dose which is well within recommended limits. Cholesterol (CDH, New Delhi) 1% was mixed along with the feed.

Each group of animals comprised of 6 mice. Group 1 served as control which was given only vehicle (0.1 ml distilled water orally/day/mouse.) Another group of mice was fed 1% cholesterol w/w for 100 days without or with myrobalan (group 2 and 3 respectively).

Mean values of chosen parameters from a large group of mice were recorded on zero day i.e. on the onset day of experimentation which were used as base line for comparison, hence initial values are same in all groups. This large group of mice were randomly divided in to above mentioned three subgroups (Gr.I, Gr.II & Gr.III).

The mice were weighed on zero days i.e. at the start (initial value) and at the end of the experiment (final value). Food intake was determined by weighing the leftover food. After an overnight fast, the animals were anesthetized and blood was drawn by heart puncture. Serum cholesterol and triglycerides were determined using the kit method of Accurex Biomedical Pvt. Ltd., Thane, India. Experiments were performed in duplicate. Sections of aorta, 5 mm thick, were fixed using Bouin's solution and stained using hematoxylin eosin. The percentage area of wall and lumen of aorta was measured using camera lucida drawings. Mean values of wall and lumen of aorta were obtained from five sections.

The statistical analysis was performed by students 't' test and the values less than 5% were considered significant.

RESULTS

Cholesterol feeding to mice (group 2) resulted in an *increased body weight as compared to controls* (table 1, group 1). Myrobalan administration to cholesterol fed mice (group 3) showed decrease in body weight as compared to cholesterol fed animals (group 2)

Cholesterol feeding (group 2) to mice caused higher food intake as compared to control (group1) animals. Myrobalan administration to cholesterol fed mice

(group 3) also resulted in higher food intake than control.

Cholesterol feeding to mice (group 2) resulted in an increased serum cholesterol, serum triglyceride and area of wall of aorta and decreased lumen in comparison to the control (group 1), myrobalan administration to cholesterol fed mice (group 3) significantly reversed these effects of cholesterol feeding

DISCUSSION

The atherogenic diet fed to mice increased the food intake, which resulted in the increased body weight of the animals. Atherodiet elevated serum cholesterol and triglyceride levels (Table 1). Myrobalan treatment to cholesterol fed animals significantly decreased serum cholesterol, triglycerides, as well as the body weight. However, no significant decrease in food intake was observed in this group (group 3).

It is probable that the synthesis and degradation of cholesterol is altered by myrobalan treatment. Myrobalan consists of saponins, which have been shown to prevent cholesterol absorption as they interfere with the enterohepatic circulation and increases its excretion^{4,5}. Myrobalan inducts gastric motility and also enhances gastric emptying⁶ which may contribute to more excretion of cholesterol resulting in decreased serum cholesterol levels. A herbal drug containing *T. chebula* was observed to be effective against CC1⁴- induced hepatopathy and hematological changes⁷. Some reports suggest that tannins increased faecal bile excretion⁴. As myrobalan possesses both saponins and tannins, it is possible that absorption of cholesterol decreased with increase in cholesterol

excretion through bile, in myrobalan fed animals (group 3).

The leaves of *T. chebula* were found to exert hypolipidemic action through inhibition of cholesterol biosynthesis and increased faecal bile excretion⁸. It is also observed that *T. chebula* enhanced lecithin : cholesterol acyl transferase (LCAT) activity⁸, which is important in the elimination of cholesterol ester, which diffuses into the core of HDL particles. Myrobalan may thus decrease the synthesis of cholesterol and also eliminate it through bile or metabolize it to other non-harmful components.

Flavanoids have been reported to have antihyperlipidemic properties^{9,10}. Some terpenoids were shown to inhibit LPO¹¹. Naturally occurring triterpenoids and some of their derivatives are known to have

hypolipidemic activity^{12,13}. Antioxidant action of *T. chebula* has also been suggested¹⁴. The presence of flavanoids and terpenoids in myrobalan may thus lower the serum cholesterol and triglyceride levels in group 3 animals.

It is concluded that myrobalan lowers serum cholesterol and triglyceride levels probably by altering synthesis, degradation and elimination of cholesterol.

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Table 1 : Effect of Myrobalan on various parameters in mice fed atherogenic diet

S. No.	Parameters		Group of Mice		
			1 (Controls)	2 (Atherodiet)	3 (Atherodiet + Myrobalan)
01	Body Weight (gm)	Initial	25.65 ± 0.46	25.65 ± 0.46	25.65 ± 0.46
		Final	28.12 ± 0.33*	30.48 ± 0.53 * ^b	28.32 ± 0.28* ^c
		% Change	(9.62)	(18.83)	(10.40)
02	Food Intake (gm/day/mouse)	Initial	5.61 ± 0.56	5.61 ± 0.56	5.61 ± 0.56
		Final	7.51 ± 0.47*	9.15 ± 0.55 * ^b	9.21 ± 0.61 * ^a
		% Change	(33.86)	(63.10)	(64.17)
03	Serum Cholesterol (mg%)	Initial	126.05 ± 3.61	126.05 ± 3.61	126.05 ± 3.61
		Final	128.96 ± 2.77	178.50 ± 188 * ^b	148.77 ± 3.14* ^{a,c}
		% Change	(2.30)	(41.61)	(18.02)
04	Serum Triglyceride (mg%)	Initial	105.61 ± 3.89	105.61 ± 3.89	105.61 ± 3.89
		Final	108.14 ± 3.15	141.11 ± 3.37 * ^b	119.44 ± 2.80* ^{a,c}
		% Change	(2.36)	(33.61)	(13.07)
05	Ascending aorta Wall	Initial	15.02 ± 1.48	15.02 ± 1.48	15.02 ± 1.48
		Final	15.11 ± 1.62	27.45 ± 1.08 * ^b	19.60 ± 1.13 * ^{a,c}
			(0.59)	(82.75)	(30.49)
	(% area of) & Lumen	Initial	84.98 ± 1.11	84.98 ± 1.11	84.98 ± 1.11
		Final	84.89 ± 1.01	72.55 ± 1.23 * ^b	80.40 ± 1.01 * ^{a,c}
			(0.10)	(14.62)	(5.38)

Values are mean ± SEM of 6 animals. a,b,c are p value (0.05), a,b,c refer to when group 3 was compared to group 1, group 2 was compared to group 1 and group 3 was compared to group 2 respectively. Value in parenthesis indicate % change.

*- Significant difference between initial and final changes when values were compared within a group only.