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Hypolipidemic and Antiatherosclerotic Effect of *Leptadenia pyrotechnica* Extract in Cholesterol Fed Rabbits

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Abstract : The aim of the present study was to evaluate the hypolipidemic and antiatherosclerotic efficacy of methanolic extract of the aerial part of *L. pyrotechnica* in cholesterol fed rabbits. Male rabbits were randomly assigned to three groups: Groups I normal control; Group II cholesterol (500 mg / kg b. wt. / day) with coconut oil (5 ml/rabbit / day) and Group III , cholesterol + *L. pyrotechnica* extract (250 mg / kg b. wt./ day) orally. The whole study lasted for 60 days. Administration of cholesterol caused a significant rise ($p<0.001$) in the serum levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and total cholesterol, : HDL-cholesterol and LDL-cholesterol : HDL-cholesterol ratios. Simultaneous administration of *L. pyrotechnica* extract significantly ($p<0.001$) prevented the rise in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and atherogenic index Hepatic and aortic total cholesterol, triglycerides, and lipid peroxidation (TBARs) were also lowered significantly in the extract treated rabbits. The Plant extracts also significantly prevented the atheromatic changes and plaque formation in the aorta and favoured increased fecal cholesterol output. Thus, the results indicate hypolipidemic and antiatherosclerotic effect of methanolic extract of *L. pyrotechnica*.

Key words : *Leptadenia pyrotechnica*, Hypolipidemic, Antiatherosclerotic, Rabbits.

Introduction :

Coronary heart disease resulting from progressive atherosclerosis, remains the most common cause of morbidity and mortality all over the world (Yusuf *et al*., 2001). In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of a cardiovascular epidemics (Grover *et al*., 2003; Okrainec *et al*, 2004). The circulatory system disorders are going to be the greatest killer in India by the end of year 2015 (Kaul *et al*., 1998). It is well established that increased levels of blood cholesterol especially low density lipoprotein cholesterol (LDLc) is an important risk factor for cardiovascular complications since it favours lipid deposition in tissues including blood vessels. Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDLc) is associated with decreased risk of atherosclerosis and coronary heart disease. (Brown *et al.,* 1998; Grundy *et al.,* 2004). Furthermore, Epidemiological studies have also shown an inverse correlation between high density lipoprotein cholesterol (HDLc) level and the risk of cardiovascular disease (Wierzbicki, 2005).

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A large number of allopathic hypolipidemic drugs are currently available in the market but these lag behind the desired properties such as efficacy and safety on long term use, cost and simplicity of administration. These factors do not fulfill conditions for patients compliance (Davidson and Tooth, 2004). Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects (Wang and Ng, 1999; Dwivedi., 2004).

Leptadenia pyrotechnica (Forsk) Decne (Asclepiadaceae) locally known as khinp is a plant occurring in wild in sandy places in Punjab, Western Utter Pradesh, Rajasthan and along sea coast in Bombay (Rastogi and Mehrotra, 1984). In Ayurvedic system of medicine, the plant is considered as analgesic, anti-inflammatory, anabolic, astringent and laxative (Pandey, 2001). The pods of this plant are used as food by Meena tribes in Rajasthan (Baghal *et al*., 2002) . The plant has been reported to be rich in alkoloids, β-sitosterol and polyhydroxy pregnane glycosides which possess cytotoxic activity against various tumor cell line (Rastogi and Mehrotra, 1984; Cioffi *et al*., 2006). In the present study, an attempt was made to elucidate the hypolipidemic and antiatherosclerotic efficacy of methanolic extract of the aerial parts of L. pyrotechnica in cholesterol fed rabbits.

Materials and methods :

Preparation of extract: Aerial parts of *L. pyrotehnica* (Forsk) Decne. (Asclepiadaceae) were collected and authenticated at the Herbarium of Botany Department, University of Rajasthan, Jaipur and a voucher specimen was deposited (RUBL No. 19876). The plant material was dried in shade, coarsely powdered and extracted with methanol at 70-80^{o}C for 30 h in soxhlet apparatus. The extract was filtered and evaporated to dryness under low temperature and reduced pressure. The crude extract so obtained was used for experimental study.

Animals : Adult, healthy, male albino rabbits, weighing 1.2 - 1.5 kg. were used in the study. Animals were housed individually in standard metallic wire gauge cages at 24±3^{o}C with 12 h light-dark cycle and provided standard laboratory feed and water *ad libitum*. After proper acclimatization for 10 days they were divided into three groups of five animals each. Animals of Group I were fed on normal standard rabbit chow. Animals of Group II and III were made hypercholesterolemic by feeding them in addition to the normal diet, 0.5 g/kg b.wt. Cholesterol powder mixed in 5 ml coconut oil daily for 60 days. Group III rabbits were also treated orally with *L. pyrotechnica* extract (250 mg/kg b.wt./ day) suspended in 1 ml double distilled water simultaneously with cholesterol for 60 days.

The study was approved by the Ethical Committee of the University Department of Zoology, Jaipur, India and standard guidelines were followed for maintenance and use of the experimental animals.

Autopsy schedule: At the end of treatment period, the overnight fasted animals were sacrificed under ether anesthesia. Blood was collected directly from the heart and serum was separated and stored at -20^{o} C for biochemical estimations. Liver was removed, cleared and weighed. The aorta extending from the base of the heart to the iliac bifurcation was removed.

Serum biochemistry: Serum samples were analyzed for total cholesterol (Zlatkis *et al.*, 1953), HDL-cholesterol (Burnstein *et al.*, 1970) and triglycerides (Gottfried and Rosenberg, 1973). LDL-cholesterol and VLDL-cholesterol were calculated by method of Friedwald *et al* (1972). Serum total cholesterol: HDL cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio were also calculated to access the atherogenic risk.

Tissue biochemistry: Biochemical analysis of fresh / frozen (-70^{o} C) liver and aorta samples were conducted for total cholesterol (Zlatkis *et al.*, 1953), triglycerides (Gottfried and Rosenberg,1973) and lipid peroxidation (TBARs) (Ohkawa *et al*., 1979).

Fecal cholesterol: Fecal samples were collected from individual rabbit during the last 7 days of treatment. These fecal samples were dried at 40^{o}C, homogenized and extracted with chloroform: methanol (2:1 ratio) (Folch *et al*., 1957). The extract was dried and used for cholesterol determination (Zlatkis *et al*., 1953).

Histological analysis : For histopathological examinations, aorta was fixed in calcium formol solution. Paraffin-embeded tissue section (5μm) were prepared and stained with hematoxylin and eosin. Planimetry studies of cross sections of aorta were made with the help of camera lucida drawings at x20.

Statistical analysis : All the results are presented as mean ± SEM. Statistical evaluation was done with Students "t" test. Differences were considered to be statistically significant at $P<0.05$ level.

Results :

Table 1 shows the values of serum lipid profile in normal, cholesterol fed control and extract treated groups. Serum total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides levels increased significantly ($P<0.001$) after 60 days of cholesterol feeding. Furthermore, the total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio were also increased significantly ($P<0.001$). Concurrent administration of *L. pyrotechnica* extract (250 mg / kg b.wt./day) with cholesterol caused a significant decrease ($P<0.001$) in the levels of serum total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triglycerides when compared with cholesterol fed control rabbits. The ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol were also declined significantly ($P<0.001$) as compared to cholesterol fed control rabbits.

Cholesterol, triglycerides and lipid peroxidation (TBARs) levels in both liver and aorta showed a significant ($p<0.001$) increase in cholesterol fed rabbits. Co-treatment of *L. pyrotechnica* extract with

cholesterol caused a significant prevention in the rise of these parameters in liver and aorta (Table 2).

Planimetric study of aorta in normal rabbits did not show any atherosclerotic plaque formation. The aorta of cholesterol fed rabbits showed the presence of large atherosclerotic plaque protruding in the lumen covering 29.35% area. Administration of *L. pyrotechnica* extract caused a significant amelioration in the atherosclerotic lessions as evidenced by significant reduction ($P<0.001$) in the plaque size (12.34%) and consequently an increase ($P<0.01$) in the lumen area (Table 3).

Discussion :

It is well documented that elevated total cholesterol and low density lipoprotien cholesterol (LDLc) levels promote atherosclerosis and cardiovascular complications (Dominiczak, 1998). Oxidative modification of low density lipoprotein cholesterol (LDLc) appears to have an important role in initiation and progression of atherogenic changes in aorta (Esterbauer *et al.,* 1993). The agents which can lower serum cholesterol and scavenge or inhibit free radicals formation have gained wide therapeutic value.

Cholesterol feeding in rabbits caused a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDL-cholesterol, and also in the ratios of total cholesterol:HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. These results are consistent with earlier reports (Prasad, 2005; Vijaimohan *et al.,* 2006) which have clearly established a correlation between dietary lipids and serum lipid profile. Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL by the liver and intestine (Demacker *et al.,* 1991) and a reduced number as well as rate of cholesterol removal by the hepatic LDL receptors (Goldstein *et al.,* 1983). Consequently serum levels of LDL-cholesterol and VLDL-cholesterol is increased. A significant increased in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol indicate increased risk of atherosclerosis and coronary heart disease (Ram, 1996).

Simultaneous Administration of *L. pyrotechnica* extract caused a significant decrease in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol suggesting beneficial modulatory influence on cholesterol metabolism and turnover. Decline in the ratios of total cholesterol : HDL-cholesterol and LDL-cholesterol : HDL-cholesterol observed in the extract treated rabbits might be a consequence of higher proportion of HDL-cholesterol which reduced atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to liver (Kinosian *et al.,* 1994; Hermansen *et al.,* 2003). Elevated serum triglycerides is considered as independent risk factor for cardiovascular disease (Asia Pacific Cohort Studies Collaboration, 2004). A significant decline in the serum triglycerides level observed in plant extract treated rabbits supports the cardiovascular protective influence.

Table 1 : Effect of *L. pyrotechnica* extract on serum lipid profile in rabbits

Treatment	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)	Triglycerides (mg/dl)	Tc/HDLc ratio	LDL-c/HDLc ratio
Normal control	90.20 ± 6.87	37.83 ± 4.70	38.0 ± 2.50	14.36 ± 0.68	71.81 ± 3.43	2.37 ± 0.12	0.99 ± 0.44
Cholesterol fed control	1345.60± 114.21 [a]	1177.88 ± 106.63 [a]	112.20 ± 6.83 [a]	55.72 ± 5.36 [a]	278.60 ± 26.83 [a]	11.99 ± 0.71 [a]	10.47±0.67 [a]
Cholesterol + *L. pyrotechnica* extract (250mg/ kg b.wt)	509.00± 41.32 [***]	399.20 ± 36.40 [***]	80.61 ± 5.63 [***]	29.08 ± 1.42 [***]	145.04±7.11 [***]	6.31 ± 0.40 [***]	4.96±0.39[***]

Tc = Total cholesterol, HDLc = HDL - cholesterol, LDLc = LDLcholesterol, Values are mean ± SEM (n=5)

Levels of significance

[a] P<0.001 when compared with normal control rabbits

[***] P < 0.001 when compared with cholesterol fed control rabbits.

Table 2 : Effect of *L. pyrotechnica* extract on liver and aortic total cholesterol, triglycerides, lipid peroxide (TBARs) and fecal cholesterol levels in rabbits

Treatment	Total cholesterol (mg/g)		Triglycerides (mg/g)		Lipid peroxide (TBARs) (n mole /g)		Fecal cholesterol (mg/g)
	liver	aorta	liver	aorta	liver	aorta	
Normal control	9.00 ± 0.28	7.11 ± 0.65	8.32 ± 0.47	7.80 ± 0.86	12.20 ± 1.51	11.18 ± 1.27	44.94 ± 2.63
Cholesterol fed control	22.35 ± 1.62 [a]	19.90 ± 1.04 [a]	19.64 ± 1.11 [a]	17.19 ± 1.30 [a]	38.61 ± 3.51 [a]	37.05 ± 3.64 [a]	81.14 ± 4.16 [a]
Cholesterol + *L. pyrotechnica* extract (250mg/ kg b.wt)	14.36± 1.16 [**]	13.13 ± 0.96 [**]	14.70 ± 1.02 [**]	13.02 ± 1.54 [*]	25.19 ± 1.99 [**]	22.31 ± 2.31 [**]	108.28 ± 4.91[**]

Levels of significance Values are mean± SEM (n=5)

[a] P<0.001 when compared with normal control rabbits

[*] P < 0.05 ; [**] P < 0.01 when compared with cholesterol fed control rabbits.

Table 3 : Effect of *L. pyrotechnica* extract on planimetric dimensions of the aortic arch in rabbits.

Treatment	Lumen area (%)	Plaque are (%)	Total wall area (%)
Normal control	53.73	Nil	45.16 ± 2.98
Cholesterol fed control	37.24 ± 2.43 [a]	29.35 ± 1.43 [a]	65.84 ± 3.36 [a]
Cholesterol + *L. pyrotechnica* extract (250mg/kg b.wt)	50.65 ± 3.00 [**]	12.34 ± 2.22 [***]	52.04 ± 2.76 [**]

Values are mean ± SEM (n=5)

Levels of significance

[a] P<0.001 when compared with normal control rabbits

[**] P < 0.01 ; [***] P< 0.001 when compared with cholesterol fed control rabbits.

Cholesterol feeding caused a considerable accumulation of cholesterol and triglycerides in liver and aorta both. These results are supported by many earlier studies (Mehta *et al.*, 2003; Purohit and Vyas, 2006). Total cholesterol content of the aorta is a good indirect measure of atherosclerotic severity in cholesterol fed rabbits (Nielson *et al*., 1993). Simultaneous administration of *L. pyrotechnica* extract caused a significant decline in the cholesterol and triglycerides contents in both liver and aorta indicating hypolipidemic effect.

The cholesterol lowering effect of the plant extract is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids.(Purohit and Vyas,2006).

A significant elevation in the level of lipid peroxide (TBARs) in both liver and aorta of cholesterol fed rabbits indicates enhanced oxidative stress in hyperlipidemic state which implicates in development and progression atherosclerotic lesions in aorta (Prasad, 2005). Administration of plant extract decreased lipid peroxidation in these tissues indicating antioxidant like activity which alleviates oxidative stress. The result of planimetric study of aortic arch also supports the hypolipidemic and antiatherosclerotic activity of the plant extract.

From these result it can be concluded that methanolic extract of aerial parts of *L. pyrotechnica* contains active components which decreases serum lipid profile and lowers the risk of atherosclerosis in cholesterol fed rabbits. Further, studies are required to gain more insight in to the possible mechanism of action.

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