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Antihyperlipidemic Activity of Various Extracts from Root of *Clerodendrum phlomidis* (Linn.) in Rat Fed with High Fat Diet

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The aim of the present study was to investigate the effect of the various extract from root of *Clerodendrum phlomidis* in reducing the cholesterol levels in experimentally induced hyperlipidemic rats. The elevated levels of total cholesterol, ester and free cholesterol, phospholipids, triglycerides, low-density lipoprotein and very low-density lipoprotein due to high fat diet. Administration of ethanolic extract of *Clerodendrum phlomidis* at the dose of 300 mg/kg was significantly ($p < 0.001$) reduced the lipid and lipoprotein profile when compared with other extracts treatment groups. A significant ($p < 0.001$) reduction in HDL-cholesterol was noticed in high fat diet fed groups (II); however, a significant ($p < 0.001$) increased the HDL level was produced by the administration of ethanolic extract of *Clerodendrum phlomidis* (dose 300 mg/kg). There was a noticed increase in the body weight in high fat diet fed group (II), which was reduced by the administration of ethanolic extract of *Clerodendrum phlomidis* (dose 300 mg/kg). Therefore, it was concluded that the ethanolic extract of root of *Clerodendrum phlomidis* has definite cardio protective effect against hyperlipidemia.

Key Words: *Clerodendrum phlomidis*, High fat diet, Rats, Hyperlipidemia.

INTRODUCTION

Atherosclerosis, the most important pathologic process leading to cardio and cerebrovascular diseases, is suggested to be mediated by the increase in the serum lipid, thrombosis and injuries of the endothelial cells[1,2]. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol established[3]. Some of the major limitations in the effective pharmacological treatment of hyperlipidemia are the constraints imposed on health care resources, particularly in the low-and middle-income countries[4]. There is a need to tackle this physiological problem as it is attaining grave proportions globally. In this scenario, the problem may be tackled by use of natural agents due to their cost effectiveness and minimal side-effects[5]. In recent times, much research interest has been focused on various herbs that possess hypolipidemic properties that may be useful in reducing the risk of cardiovascular diseases[6].

Clerodendrum phlomidis, Linn. f. suppl. is belonging to the family Verbanaceae, which is mentioned under the common name of Arni and/or Agnimantha in Ayurveda[7]. Their roots are important ingredient of Ayurvedic preparations like Dashmoolakwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaaktel, Narayan tel *etc*., valued for the treatment of variety of ailments[8]. *C. phlomidis* roots are valued as tonic, diuretic, febrifuge, antidiabetic, antiinflammatory, antidiahhoreal and antitussive[9-11]. Phytochemical studies include presence of b-sitosterol and g-sitosterol, ceryl alcohol, clerodin, clerosterol, clerodendrin-A[12] and flavanoids, pectolinarigenin, hispidulin, apigenin luteolin[13]. The objective of the present study was to evaluate the antihyperlipidemic activity of various extracts from root of *Clerodendrum phlomidis* in rat fed with high fat diet.

EXPERIMENTAL

Plant materials and preparation of extracts: The roots of *Clerodendrum phlomidis*, were collected from Chennai, Tamil Nadu, India. The plant material was identified by Dr. Sasikala Ethirajulu, Research officer, CCRAS, Govt. of India, Chennai. The roots of *Clerodendrum phlomidis* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The dried powder of the roots was extracted sequentially by hot continuous percolation method using Soxhlet apparatus[14], using different polarities of solvents like petroleum ether, chloroform, ethyl acetate and ethanol. The dried plant material was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 h. Then the marc was subjected to chloroform for 24 h and the marc was subjected to ethyl acetate for 24 h and then marc was subjected to ethanol for 24 h. The extracts were concentrated by using a rotary evaporator and subjected to

freeze drying in a lyophilizer till dry powder was obtained. Then the extracts were suspended in 2 % Tween 80[15].

Animal diet: The compositions of the two diets were as follows[16]; control diet: wheat flour 22.5 %, roasted bengal gram powder 60 %, skimmed milk powder 5 %, casein 4 %, refined oil 4 %, salt mixture with starch 4 % and vitamin and choline mixture 0.5 %. High fat diet: wheat flour 20.5 %, roasted bengal gram 52.6 %, skimmed milk powder 5 %, casein 4 %, refined oil 4 %, coconut oil 9 %, salt mixture with starch 4 % and vitamin and choline mixture 0.5 %, cholesterol 0.4 %.

Experimental design: Male Wister rats of 16-19 weeks age, weighing 150-175 g were procured from the Central Animal House, Madras Medical College, Chennai. The animals were kept in cages, 2 per cage, with 12:12 h light and dark cycle at 250 ± 2 ºC. The animals were maintained on their respective diets and water ad libitum. Animal ethical Committee's clearance (6/243/cpcsea) was obtained for the study. Animals were divided into following 7 groups of 6 animals each: Group I (control) : Standard chow diet. Group II: High fat diet. Group III: High fat diet + pet ether extract of *Clerodendrum phlomidis*. Group IV : High fat diet + chloroform extract of *Clerodendrum phlomidis*. Group V: High fat diet + ethyl acetate extract of *Clerodendrum phlomidis.* Group VI: High fat diet + ethanolic extract of *Clerodendrum phlomidis*. Group VII : High fat diet + standard drug atorvastatin.

Rats of groups III, IV, V and VI were orally fed with the various extracts of *Clerodendrum phlomidis* at the dose of 300 mg/kg b.wt and rats of group VII were fed with standard drug atorvastatin at the dose of 1.2 mg/kg b.wt. Both the *Clerodendrum phlomidis* extracts and atorvastatin were suspended in 2 % Tween 80 separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the animals were sacrificed by cervical dislocation under mild anaesthesia after overnight fasting. Blood was collected in heparinized tubes and plasma was separated. Liver, heart and aorta were cleared of adhering fat, weighed accurately and used for the preparation of homogenate. Animals were given enough care as per the Animal Ethical Committee's recommendations.

Biochemical estimation: Plasma samples were analyzed for total cholesterol, HDL-cholesterol and triglycerides using Boehringer Mannheim kits by Erba Smart Lab analyzer USA. LDL-Cholesterol and VLDL-cholesterol were calculated by using Friedwald method[17]. Ester cholesterol[18] and free cholesterol[18] were analyzed by using digitonin. Portions of liver, heart and aorta tissues were blotted, weighed and homogenized with methanol (3 volumes) and the lipid extracts were obtained by the method of Folch *et al*.[19]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides[20] and phospholipids[21].

Statistical analysis: Results were expressed as mean ± SE of 6 rats in each group. One way analysis of variance (ANOVA) followed by Dunnet multiple comparison tests was used to determine the statistical significance. Significance level was fixed at 0.05.

RESULTS AND DISCUSSION

Table-1 shows the average body weight changes in control and experimental animals in each group. The average body weight of rats in all the seven groups was increased after 9 weeks of experimental period. But high fat diet fed rats (group II) had a significant ($p < 0.001$) increase in body weight compared with other group rats. After administration of various extracts of *Clerodendrum phlomidis* it was found to be decreased in body weight. But the administration of the ethanolic extract of *Clerodendrum phlomidis* were found to more significantly ($p < 0.001$) decreased the body weight when compared to high fat diet rats group (II).

TABLE-1
AVERAGE BODY WEIGHT CHANGES IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP

Groups	Initial weight (g)	Final weight (g)
Group I	206.66 ± 9.13^{bNS}	$208.33 \pm 6.14^{b**}$
Group II	209.16 ± 5.83^{aNS}	$228.33 \pm 4.59^{a**}$
Group III	$225.00 \pm 3.65^{aNS,bNS}$	$221.66 \pm 3.33^{aNS,b*}$
Group IV	$223.33 \pm 4.21^{aNS,bNS}$	$208.33 \pm 6.01^{aNS,b*}$
Group V	$233.33 \pm 1.66^{aNS,bNS}$	$213.33 \pm 1.66^{aNS,b**}$
Group VI	$226.66 \pm 4.94^{aNS,bNS}$	$196.66 \pm 4.77^{aNS,b**}$
Group VII	$224.16 \pm 6.63^{aNS,bNS}$	$197.50 \pm 8.34^{aNS,b**}$

Values are expressed as mean ± SE (n = 6 rats), p values: * < 0.001, **< 0.05. NS: Non significant. a: Group I compared with groups II, III, IV, V, VI. B: group II compared with groups III, IV, V, VI.

Table-2 shows the effect of various extracts of *Clerodendrum phlomidis* on plasma lipid profile in control and experimental rats in each group. Total cholesterol levels were increased in high fat diet fed rats (group II) as compared to control rats (group I). Results show that treatment with high fat diet significantly increased the concentration of plasma and tissue lipids as reported earlier revealing that significant elevation of plasma and tissue lipid parameters in response to atherogenic diet and

TABLE-2
EFFECT OF VARIOUS EXTRACTS OF *Clerodendrum phlomidis* ON PLASMA LIPID PROFILE IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	$111.98 \pm 1.02^{b*}$	$25.04 \pm 0.91^{b*}$	$86.94 \pm 0.92^{b*}$	$103.16 \pm 0.63^{b*}$	$80.06 \pm 0.92^{b*}$	$1.87 \pm 0.03^{b*}$
Group II	$171.67 \pm 1.74^{a*}$	$43.40 \pm 0.78^{a*}$	$128.26 \pm 1.47^{a*}$	$145.48 \pm 0.42^{a*}$	$150.12 \pm 1.17^{a*}$	$4.18 \pm 0.17^{a*}$
Group III	$153.30 \pm 3.87^{a**,b*}$	$39.74 \pm 0.97^{a*,b*}$	$120.17 \pm 2.25^{a*,b**}$	$141.79 \pm 0.51^{a*,b*}$	$140..43 \pm 1.37^{a*,b*}$	$2.76 \pm 0.04^{a**,b*}$
Group IV	$130.12 \pm 1.19^{a**,b**}$	$39.74 \pm 0.97^{a**,b**}$	$100.32 \pm 1.02^{a**,b*}$	$142.47 \pm 0.48^{a*,b*}$	$135.52 \pm 1.08^{a**,b*}$	$2.76 \pm 0.03^{a*,b*}$
Group V	$115.64 \pm 1.07^{a**,b*}$	$25.44 \pm 0.58^{a*,b**}$	$90.2 \pm 1.38^{a*,b**}$	$126.16 \pm 0.63^{a*,b*}$	$116.10 \pm 1.67^{a*,b*}$	$2.25 \pm 0.03^{a*,b*}$
Group VI	$99.63 \pm 0.51^{a*,b*}$	$22.35 \pm 0.45^{a*,b*}$	$77.28 \pm 0.72^{a*,b*}$	$108.07 \pm 0.42^{a*,b*}$	$74.41 \pm 0.96^{a*,b*}$	$1.72 \pm 0.02^{a*,b*}$
Group VII	$98.03 \pm 0.33^{a*,b*}$	$22.18 \pm 0.21^{a*,b*}$	$75.85 \pm 0.1^{b*}$	$98.58 \pm 1.17^{a*,b*}$	$74.25 \pm 0.41^{a*,b*}$	$2.16 \pm 0.01^{b*}$

Values are expressed as mean ± SE (n = 6 rats), *p* values: * < 0.001, **< 0.05. NS: Non-significant. a: Group I compared with groups II, III, IV, V, VI. B: group II compared with groups III, IV, V, VI.

cholesterol feeding[22,23]. Administration of ethanolic extract of *Clerodendrum phlomidis* (dose 300 mg/kg body weight) to rat fed with high fat diet significantly ($p < 0.001$) decreased in the concentration of total cholesterol as compared to high fat diet rats (group II). But the administration of ethanolic extract of *Clerodendrum phlomidis* treated rats with high fat diet showed that the plasma cholesterol was restored to near normal as that of atorvastatin (group VI).

Effect of free and ester cholesterol in plasma and tissue were present in Tables 2 and 4. Significant ($p < 0.001$) increase in levels of both free and ester cholesterol were also observed in plasma and tissue of rats fed with high fat diet (group II). This high cholesterol concentration in circulation may damage the endothelial cells lining the large arteries and aorta and this may be an initial event in the etiology of atherosclerosis[24]. Both plasma free and ester cholesterol reduced remarkably on treating the high fat diet rats with ethanolic extract of *Clerodendrum phlomidis*. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of fecal sterol[25].

Effect of the various extracts of *Clerodendrum phlomidis* on plasma and tissue triglyceride are presented in Tables 2 and 5. The concentration of plasma and tissue triglyceride was elevated in rats fed with high fat diet (group II) as compared to control rats (group I). HFD rats had significant increase in the level of plasma triglyceride due to decrease in the activity of lipoprotein lipase[26,27]. Both plasma and tissue triglyceride levels were significantly reduced in rats treated with ethyl acetate and ethanolic extracts of *Clerodendrum phlomidis* (300 mg/kg b.wt) and as well as standard drug atorvastatin along with high fat diet when compared with rats fed with high fat diet (group II). Administration of ethanolic extract of *Clerodendrum phlomidis* significantly reduced the triglyceride when compared with other extracts treatment groups. The plant extract may have stimulation of lipoprotein lipase activities resulting in decrease of plasma triglyceride and might increase the uptake of triglyceride from plasma by skeletal muscle and adipose tissues[28].

Effect of various extracts of *Clerodendrum phlomidis* on plasma and tissue phospholipids are presented in Tables 2 and

TABLE-3
EFFECT OF VARIOUS EXTRACTS OF *Clerodendrum phlomidis* ON PLASMA LIPOPROTEIN IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Group I	59.41 ± 0.88^{b*}	36.61 ± 1.03^{b*}	16.16 ± 0.22^{b*}
Group II	41.41 ± 1.97^{a*}	100.22 ± 1.63^{a*}	30.03 ± 0.23^{a*}
Group III	28.69 ± 0.37^{a*}	143.10 ± 0.55^{a*}	29.41 ± 0.09^{a*}
Group IV	38.38± 0.51^{a*,b*}	84.94 ± 0.26^{a*,b*}	17.42 ± 0.13^{a*,b*}
Group V	42.73 ± 0.99^{a*,b*}	54.82 ± 1.22^{a*,b**}	27.08 ± 1.32^{a*,b*}
Group VI	57.83 ± 0.71^{a*,b*}	26.78 ± 1.13^{a*,b*}	14.88 ± 0.19^{a*,b*}
Group VII	56.63 ± 0.55^{a*,b*}	27.21 ± 1.17^{a*,b*}	13.15 ± 0.14^{a*,b*}

Values are expressed as mean ± SE (n = 6 rats), p values: * < 0.001, **< 0.05. NS: Non-significant. a: Group I compared with groups II, III, IV, V, VI. B: group II compared with groups III, IV, V, VI.

TABLE-4
EFFECT OF VARIOUS EXTRACTS OF *Clerodendrum phlomidis* ON TISSUES ESTER CHOLESTEROL AND FREE CHOLESTEROL PROFILE IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP

Groups	Ester cholesterol (mg/g tissue)			Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	2.56 ± 0.12^{b*}	1.88 ± 0.02^{b*}	1.91 ± 0.02 ^{b*}	0.81 ± 0.06 ^{b*}	0.73 ± 0.02 ^{b*}	0.49 ± 0.03 ^{b*}
Group II	5.67 ± 0.07^{a*}	3.51 ± 0.10 ^{a*}	6.81 ± 0.23 ^{a*}	1.51 ± 0.03 ^{a*}	1.32 ± 0.08 ^{a*}	2.43 ± 0.17 ^{a*}
Group III	3.25 ± 0.13^{a*}	7.07 ± 0.16^{a*}	6.34 ± 0.15^{a*}	1.31 ± 0.04 ^{a*}	1.06 ± 0.04 ^{a*}	1.74 ± 0.08 ^{a*}
Group IV	2.88 ± 0.09^{a**,b*}	4.97 ± 0.12^{a*,b**}	5.65 ± 0.04^{a*,b**}	1.26 ± 0.05 ^{a**,b*}	1.00 ± 0.04 ^{a*,b*}	1.67 ± 0.03 ^{a*,b**}
Group V	2.58 ± 0.06 ^{a*,b*}	4.10 ± 0.09 ^{a*,b*}	4.98 ± 0.24 ^{a**,b*}	0.93 ± 0.01^{a*,b*}	0.90 ± 0.02 ^{a**,b*}	1.11 ± 0.02 ^{a*,b*}
Group VI	1.90 ± 0.07 ^{a*,b*}	2.30 ± 0.05 a^{*,b**}	3.55 ± 0.06 ^{a*,b**}	0.85 ± 0.02 ^{a*,b*}	0.63 ± 0.03 ^{a*,b*}	0.70 ± 0.05 ^{a*,b*}
Group VII	1.98 ± 0.09 ^{a*,b*}	2.94 ± 0.08 ^{a*,b*}	2.83 ± 0.11 ^{a*,b*}	0.86 ± 0.04 ^{a*,b*}	0.64 ± 0.04 ^{a*,b*}	0.63 ± 0.04 ^{a*,b*}

Values are expressed as mean ± SE (n = 6 rats), p values: * < 0.001, **< 0.05. NS: Non-significant. a: Group I compared with groups II, III, IV, V, VI. B: group II compared with groups III, IV, V, VI.

TABLE-5
EFFECT OF VARIOUS EXTRACTS OF *Clerodendrum phlomidis* ON TISSUES TRIGLYCERIDE AND PHOSPHOLIPIDS LEVELS IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP

Groups	Triglyceride (mg/g tissue)			Phospholipids (mg/g tissue)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	12.40 ± 0.08^{b*}	13.69 ± 0.07^{b*}	11.70 ± 0.07^{b*}	17.52 ± 0.23^{b*}	23.50 ± 0.27^{b*}	8.81 ± 0.10^{b*}
Group II	29.47 ± 0.09^{a*}	48.24 ± 0.17^{a*}	25.19 ± 0.06^{a*}	29.63 ± 0.09^{a*}	37.41 ± 0.12^{a*}	16.64 ± 0.09^{a*}
Group III	28.56 ± 0.16^{a*}	48.24 ± 0.17^{a*}	22.14 ± 0.19^{a*}	25.81 ± 0.24^{a*}	36.06 ± 0.29^{a*}	16.31 ± 0.09^{a*}
Group IV	27.75 ± 0.13^{a**,b*}	42.87 ± 0.10^{a*,b**}	21.09 ± 0.16^{a*,b**}	25.85 ± 0.05^{a**,b*}	34.19 ± 0.15^{a*,b*}	15.24 ± 0.12^{a**,b*}
Group V	20.99 ± 0.16^{a*,b*}	37.84 ± 0.12^{a**,b*}	17.76 ± 0.09^{a*,b*}	24.61 ± 0.32^{a*,b**}	30.31 ± 0.36^{a*,b*}	13.25 ± 0.10^{a*,b*}
Group VI	15.25 ± 0.06^{a*,b*}	18.35± 0.08^{a*,b*}	14.46 ± 0.14^{a**,b*}	18.72 ± 0.17^{a*,b*}	25.39 ± 0.18^{a*,b**}	10.67 ± 0.10^{a*,b*}
Group VII	12.29 ± 0.10^{a*,b*}	21.48 ± 0.12^{a**,b*}	13.22 ± 0.12^{a*,b*}	19.21 ± 0.06^{a**,b*}	26.32 ± 0.09^{a*,b*}	10.35 ± 0.11^{a*,b*}

Values are expressed as mean ± SE (n = 6 rats), p values: * < 0.001, **< 0.05. NS: Non-significant. a: Group I compared with groups II, III, IV, V, VI. B: group II compared with groups III, IV, V, VI.

5. The concentration of plasma and tissue phospholipids were significantly increased in rats fed high fat diet (group II) as compared to control animals (group I). This may be due to decreased phospolipase activity[29,30]. After treatment of ethanolic extract of *Clerodendrum phlomidis* along with high fat diet phospholipids levels were significantly reduced as compared to high fat diet fed rats (group II). The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases[16].

Table-3 shows the levels of HDL cholesterol in plasma of control and experimental rats in each group. The HDL cholesterol levels increased in high fat diet rats (Group II) as compared to control rats (group I). But the administration of ethanolic extract of *Clerodendrum phlomidis* was found significantly elevated the HDL-cholesterol levels when compared with other extracts treatment groups. Several studies show that an increase in HDL -cholesterol is associated with a decrease in coronary risk[31].

Effect of various extracts of *Clerodendrum phlomidis* on plasma LDL and VLDL levels are presented in Table-3. HFD fed rats (group II) are elevated levels of LDL and VLDL-cholesterol when compared with the control (group I). High levels of LDL and VLDL-cholesterol are major risk factor for coronary heart disease[32]. Studies show that both LDL and VLDL have a positive role in atherogenesis[33]. Administration of ethanolic extract of *Clerodendrum phlomidis* markedly reduced the level of LDL, VLDL-cholesterol in plasma when compared with other extracts. Reduced levels of LDL and VLDL in ethanolic extract of *Clerodendrum phlomidis* on HFD fed rats may be possibly due to increase with catabolism of LDL.

Atherogenic index is used as a marker to assess the susceptility of atherogenesis. It was markedly increased on feeding high fat diet to rats. The ethanolic extract of *Clerodendrum phlomidis* significantly ($p < 0.001$) decreased atherogenic index when compared with other extracts treatment groups. But, when the ethanolic extract of *Clerodendrum phlomidis* was compared with standard group of rats were found to have similar result.

Conclusion

From these results it can be concluded that ethanolic extract of *Clerodendrum phlomidis* contains active components which decreases plasma lipid profile and lowers the risk of atherosclerosis in high fat diet. The phytoconstituents may be responsible for the hypolipidemic activities of ethanolic extract of *Clerodendrum phlomidis*. Therefore, further studies are needed to isolate the active components from this plant.

REFERENCES

1. P.J. Goldschmidt-Clermont, M.A. Creager, D.W. Losordo, G.K. Lam, M. Wassef and V.J. Dzau, *Circulation*, **112**, 3348 (2005).
2. D. Pratico, *Atherosclerosis*, **181**, 215 (2005).
3. Lipid Research Clinics Program, *J. Am. Med. Asso.*, **251**, 365 (1984).
4. M.B. Bergman, K. Kranjcevic, Z. Reiner, B.S. Milakovic, S. Stojanovic and S. Pehar, *Croat. Med.*, **46**, 984 (2005).
5. O.A. Adaramoye, O. Akintayo, J. Achem and M.A. Fafunso, *Vasc. Health Risk Manag.*, **4**, 235 (2008).
6. W.J. Craig, *Am. J. Clin. Nutr.*, **70**, 491 (1999).
7. B.G. Vaidya and N. Adarsh, (Uttarardh), Shri Swami Atamanand Sarsavati Ayurvedic, Government Pharmacy Ltd., Surat, pp. 815-820 (1965).
8. Anonymous, The Ayurvedic Pharmacopoeia of India Government of India. Ministry of Health and Family Welfare, Dept. of ISM and Homoeopathy, New Delhi Part 1, edn. 1, Vol. 3 (2001).
9. K.M. Nadkarni and A.K. Nadkarni, Indian Materia Medica, Popular Prakashan Private Ltd, Bombay, edn. 3, pp. 353-354 (1982).
10. G.N. Chaturvedi, P.R. Subraminiyam, S.K. Tiwari and K.P. Singh, *Ancient Sci. Life*, **3**, 216 (1984).
11. S.N. Rani, S.R. Ahamed Rajaram, S. Saluja, Thenmozhi and T. Murugesan, *J. Ethnopharmacol.*, **68**, 315 (1999).
12. K.C. Joshi, P. Singh and A. Mehara, *Planta Med.*, **37**, 64 (1979).
13. K.K. Seth, V.B. Pandey and B. Dasgupta, *Pharmazie*, **37**, 74 (1982).
14. J.B. Harborne, Phytochemical methods, In Chapman &, Hall.New York, edn. 11, pp. 4-5 (1984).
15. B.H. Waynforth, Injection Techniques, Academic Press, London, 3 (1980).
16. A. Kottai Muthu, S. Sethupathy, R. Manavalan and P.K. Karar, *Ind. J. Exp. Biol.*, **43**, 522 (2005).
17. W.T. Freidewald, R.I. Levy and D.S. Frederickson, *Clin. Chem.*, **18**, 499 (1972).
18. W.M. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 97 (1950).
19. J. Folch, M. Lees and G.H. Sloane, *J. Biol. Chem.*, **226**, 497 (1957).
20. C.S. Foster and O. Dunn, *Clin. Chem.*, **19**, 338 (1973).
21. B. Zilversmit and A.K. Davis, *J. Lab. Clin. Inv.*, **35**, 155 (1950).
22. R. Chandar, A.K. Khanna and N.K. Kapoor, *Phyt Res.*, **10**, 508 (1996).
23. S. Guido and T. Joseph, *Indian J. Exp. Biol.*, **30**, 292 (1997).
24. B. Hennig and C.K. Chow, *Free Radic. Biol. Med.*, **4**, 99 (1998).
25. A. Purohit and K.B. Vyas, *Atherosclerosis*, **179**, 269 (2006).
26. R. Kavitha and N. Nalini, *Med. Sci. Res.*, 17 (2001).
27. M. Van Heek and D.B. Zilversmith, *Atherosclerosis*, **71**, 185 (1998).
28. M.A. El-Hazmi and A.S. Warsy, *J. Trop. Pediatrics*, **47**, 181 (2001).
29. S.A. Mirhadi and S. Singh, *Indian J. Exp. Biol.*, **29**, 162 (1991).
30. A.F. Whereat and J.L. Robinowit, *Am. J. Cardiol.*, **55**, 567 (1975).
31. D. Harrison, K.G. Kathy, B. Hornig, H. Drexler, *Am. J. Cardiol.*, **91**, 7A (2003).
32. E.H. Temme, H.P.G. Van, E.G. Schouten and H. Kesteloot, *Acta Cardiol.*, **57**, 111 (2002).
33. S. Parthasarathy, M.T. Quinin, D.C. Schwenke, T.E. Carew and B. Steinberg, *Atherosclerosis*, **9**, 398 (1989).