Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

ANTIHYPERLIPIDEMIC ACTIVITY OF *BAMBUSA BAMBOS* (DRUCE.) AND *SWERTIA CHIRATA* (BUCH-HAM) IN CHOLESTEROL SUSPENSION INDUCED HYPERCHOLESTEROLEMIA IN RATS

SWATI R DHANDE[a*], AAKRUTI A KAIKINI[a], KALPANA A PATIL[a], VILASRAO KADAM[a]

[a] Bharati Vidyapeeth's College of Pharmacy, Sector-8, C.B.D. Belapur, Navi Mumbai-400614, Maharashtra, India.
Email: bvppharmacology@gmail.com

Received: 01 Nov 2013, Revised and Accepted: 22 Nov 2013

ABSTRACT

Objective: The objective of the present study was to investigate the antihyperlipidemic activity of methanolic extract of *Bambusa bambos* and ethanolic extract of *Swertia chirata.*

Method: Antihyperlipidemic activity was determined using cholesterol suspension induced hypercholesterolemia in rats. Rats were randomly assigned to five groups: Group I (Vehicle control), Group II (Disease control) 25mg/kg cholesterol in coconut oil, Group III (Standard control) 25mg/kg cholesterol in coconut oil + 10mg/kg Atorvastatin, Group IV (MEBB) 25mg/kg cholesterol in coconut oil + 300mg/kg methanolic extract of leaves of *Bambusa bambos* (MEBB),Group V (EESC) 25mg/kg cholesterol in coconut oil + 400mg/kg ethanolic extract of *Swertia chirata* (EESC). Cholesterol suspension and test drugs were administered for period of 28 days. At the end of experimental period blood was withdrawn from retro-orbital plexus and was used for biochemical estimation of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C). Liver was analysed for antioxidant parameters (lipid peroxidation, reduced glutathione and catalase).

Results: Administration of methanolic extract of *Bambusa bambos* and ethanolic extract of *Swertia chirata* significantly ($p<0.01$) decreased serum TC, TG, LDL-C, VLDL-C levels, atherogenic indices and significantly ($p<0.01$) increased serum HDL-C level. Lipid peroxidation was significantly ($p<0.01$) decreased whereas reduced glutathione and catalase levels were significantly ($p<0.01$) increased in liver.

Conclusion: Methanolic extract of *Bambusa bambos* and ethanolic extract of *Swertia chirata* demonstrated effective hypocholesterolmic action together with antioxidant effect.

Keywords: *Bambusa bambos, Swertia Chirata,* Antihyperlipidemic, Atherogenic indices,

INTRODUCTION

Hyperlipidemia is characterised by abnormally high levels of fatty substances called lipids, mainly cholesterol and triglycerides in blood [1]. It is a major risk factor for atherosclerosis and atherosclerosis associated conditions, such as Coronary Heart Disease (CHD), Ischemic Cerebrovascular Disease and peripheral vascular disease [2]. Presently existing hypolipidemic drugs are associated with many side effects such as myosistis, abnormal liver function, diarrhoea, flushing, hyperuricemia, gastric irritation and dry skin [3]. Therefore it is essential to focus on herbal drugs which might have a role in treatment of hyperlipidemia. *Bambusa bambos* Druce. (Family: Graminae) is claimed to be *medhoghna* i.e. removing or destroying excessive fat. According to Ayurvedic text, fruit and seeds act on *medhadhatu* and are useful in fat metabolism and obesity [4]. Charakha prescribed decoction of leaves or seeds in treatment of excessive fat [5]. The herb *Swertia chirata* Buch-ham. (Family: Gentianaceae) commonly known as Chirata' is well reputed for its multifarious therapeutic values since the era of 'Atharvaveda'. In Ayurvedic literature it is termed as Cholagugue [4, 6], meaning to promote the flow of bile which is major route for excretion of cholesterol. The herb also contains chemical constituents such as mangiferin [7] and swertiamarin [8] both known to possess antihyperlipidemic activity. Though the plants have been used extensively in traditional medicine, no such scientific evidence for antihyperlipidemic activity is available in established scientific journals of repute. Hence the present study is aimed at investigating the antihyperlipidemic potential of leaves of *Bambusa bambos* and aerial parts of *Swertia chirata.*

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Bambusa bambos* (4kgs) were procured from Keshav Shustri, Thane, India in the month of July. The leaves were authenticated by Dr. Harshad Pandit, Department of Botany, Guru Nanak Khalsa College, Mumbai, India and a voucher specimen (accession number: ak/170912) was deposited for future reference. Dried aerial parts of *Swertia chirata* (2kgs) were purchased from Yucca Enterprises, Wadala, Mumbai, India in month of July. The aerial parts were authenticated by Dr. Vinayak Pandit, Department of Botany, Piramal Life Sciences, Goregaon, Mumbai, India and a voucher specimen (accession number: PHL/6524) was deposited for further reference.

Preparation of Plant Extract

The leaves of *Bambusa bambos* were dried in shade, powdered and passed through 40 mesh sieve. Dried powder (50g) was extracted with methanol in Soxhlet apparatus for 72 Hrs. The extract was evaporated under vacuum in a rotary evaporator at a temperature of 40±0.5C and dried completely in a desiccator and weighed. The yield of the extract was 4.5g. Dried aerial parts of *Swertia chirata* were powdered and passed through 40 mesh sieve. Dried powder (400g) was subjected to cold maceration with 12% ethanol for 48Hrs with continuous stirring. The extract was concentrated under vacuum in rotary evaporator at temperature of 50±0.5C and dried completely by freeze drying to get free flowing powder. The yield of extract was 22.5g.

Formulation

For purpose of dosing, the extracts were uniformly suspended in 0.5% Sodium Carboxy Methyl Cellulose (CMC) dissolved in water and administered orally (*p.o.)*.

Experimental Animals

Adult female Sprague Dawley rats weighing 150-200g were purchased from Bharat Serums and Vaccines Ltd. Thane, Mumbai, India. The study was conducted after obtaining the clearance for the experimental protocol (IAEC/PR/2012/02) from Institutional Animal Ethics Committee (IAEC), Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, India. Rats were housed in animal house of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, India. The animal house was maintained at an average temperature (24.0±2ºC) and 30-70% RH, with 12-hr light-dark cycle (lights on 8.00a.m to 8.00p.m). Animals received humane care and were fed with

commercial pellet diet (Amrut Laboratory, Mumbai, India) and tap water *ad-libitum*. The animals were acclimatized for one week before the start of the experiment. The experiments were carried out in accordance with the guidelines set by Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

***In vivo* antihyperlipidemic activity- cholesterol suspension induced hypercholesterolemia**

The antihyperlipidemic activity was determined by the method of Dhule et al. (1999) [9]

Female Sprague Dawley rats were divided randomly into 5 groups of 6 animals each as follows:

Group I: Vehicle control group in which rats were administered daily with vehicle i.e. 0.5% w/v Sodium CMC solution

Group II: Disease control group in which the rats were administered daily with cholesterol (25mg/kg/day) in coconut oil.

Group III: Standard control group in which the rats were administered daily with Atorvastatin (10mg/kg/day) along with cholesterol (25mg/kg/day) in coconut oil.

Group IV: Test group in which the rats were administered daily with methanolic extract of leaves of *Bambusa bambos* (MEBB) (300mg/kg/day) along with cholesterol (25mg/kg/day) in coconut oil.

Group V: Test group in which rats were administered daily with ethanolic extract of *Swertia chirata* (EESC) (400mg/kg/day) along with cholesterol (25mg/kg/day) in coconut oil.

A homogeneous suspension of the extracts and standard drug Atorvastatin was freshly prepared individually using 0.5% Sodium CMC solution. Cholesterol in coconut oil (cholesterol suspension) and the test compounds were administered for period of 28 days. The time interval between administration of cholesterol suspension and test compounds was 5Hr. The vehicle control group was treated with vehicle instead of drugs.

Sample collection and evaluation

At the end of experimental period, blood was withdrawn from retro-orbital plexus following 12Hr fasting period. The blood samples were allowed to clot at room temperature for 20-25 min and centrifuged for 20 min at 3000 rpm at 4C. The supernatant clear serum thus obtained was stored at -20C until the completion of biochemical analysis. Thereafter, animals were sacrificed by CO_2 overdose and livers were excised immediately and washed with ice-cold saline. Livers were processed further for determination of antioxidant activity in liver tissue homogenate. A portion of excised liver was used for estimation of hepatic lipids [10].

Biochemical analysis

All samples were used for following biochemical investigations. The blood serum under this model was analysed for the marker parameters such as Total Cholesterol (TC), High density lipoprotein cholesterol (HDL-C) and Triglycerides (TG). Serum total cholesterol and triglycerides were estimated by enzymatic methods of CHOD-PAP [11] and GPO-Trinder method [12] respectively. Estimation of HDL-C was done by precipitation method [13]. All parameters were analysed by Auto analyser (Erba Chem7) with biochemical kit (Erba diagnostics Mannheim GmbH). Serum concentration of Very low density lipoprotein-Cholesterol (VLDL-C) and Low density lipoprotein-Cholesterol was calculated using Friedewald's formula [14]

The atherogenic indices calculated were:

Atherosclerosis Index (A.I) = LDL-C / HDL-C [15]

Cardiac Risk Ratio (C.R.R)=TC/HDL-C [16]

Atherogenic Coefficient (A.C) = TC-HDL-C/HDL-C [17]

Antioxidant activity in liver tissue homogenate

About 1g of liver tissue was homogenised in 10ml of 0.1M phosphate buffer pH 7.4 to form 10%w/v liver tissue homogenates The prepared liver tissue homogenates were centrifuged at 3500 rpm for 15 minutes and supernatant was used for the determination of various antioxidant parameters like lipid peroxidation levels [18], Reduced glutathione levels [19], and Catalase levels [20].

Estimation of hepatic lipids

Hepatic lipids were extracted using method of Folch et al. (1957) [10]. Briefly, 1gm of liver was homogenised in 20ml of solvent mixture (Chloroform: Methanol in ratio 2:1). The homogenate was filtered and washed with 4 ml of saline solution. The mixture was vortexed for few seconds and then centrifuged at 2000 rpm for separation of the two phases. The lower phase was used for estimation of hepatic lipids.

Statistical analysis

All results were expressed as mean±SEM of 6 rats in each group. The statistical significance between the groups was analysed by using one way ANOVA followed by Dunnett's multiple comparison test. Significance level was fixed at $p<0.01$

Table 1: Effect of MEBB and EESC on serum lipid profile in hyperlipidemic rats

Treatment Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Vehicle Control	63.38± 1.16	51.04± 2.07	22.07± 0.80	31.11± 1.55	10.21± 0.41
Disease Control	152.32 ±2.42	164.09 ±1.97	12.88 ±0.70	107.56 ±3.38	32.79 ±0.40
Standard Control	102.08 ±1.05** (32.98) ↓	82.25 ±1.64** (49.88) ↓	33.86 ±0.58** (162.89) ↑	51.99 ±1.24** (51.66) ↓	16.45 ±0.33** (49.83) ↓
MEBB	117.86 ±1.75** (22.62)↓	96.93 ±1.71** (40.93) ↓	28.32 ±1.13** (119.88) ↑	70.16 ±2.49** (34.77)↓	19.39 ±0.34** (40.87)↓
EESC	116.8 ±1.58** (23.31)↓	105.3 ±1.41** (35.90) ↓	26.92 ±0.62 (109.0) ↑	68.80 ±1.90 (35.46)↓	20.72 ±0.25 (35.94)↓

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett's test (p**<0.01) Values in parenthesis indicate % increase or decrease in respective parameters, ↓ indicates decrease; ↑ indicates increase.

RESULTS

Biochemical analysis

In cholesterol suspension induced hypercholesterolemia model, the groups treated with extracts of *B. bambos* (MEBB), *S. chirata* (EESC) and standard drug Atorvastatin demonstrated a significant ($p<0.01$) decrease in serum levels of total cholesterol (TC), Triglycerides (TG), LDL- Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C), besides a significant ($p<0.01$) increase in serum HDL-Cholesterol (HDL-C) levels when compared with disease control group (Table 1). Serum TC levels were reduced by 22.62%, 23.31% and 32.98% by MEBB, EESC and Atorvastatin respectively.

Serum TG levels were reduced by 40.93%, 35.90% and 49.88% by MEBB, EESC and Atorvastatin respectively. Serum LDL-C levels were reduced by 34.77%, 35.46% and 51.66 % by MEBB, EESC and Atorvastatin respectively. Serum VLDL-C levels were reduced by 40.87%, 36.94% and 49.83% respectively by MEBB, EESC and Atorvastatin respectively. Serum HDL-C levels were increased by 119.88%, 109.00% and 162.89% by MEBB, EESC and Atorvastatin respectively. The groups treated with MEBB, EESC and standard drug Atorvastatin demonstrated a significant ($p<0.01$) decrease in Atherosclerosis Index (A.I), Cardiac Risk Ratio (C.R.R) and Atherogenic Coefficient (A.C) when compared with disease control group (Table 2).

Table 2: Effect of MEBB and EESC on Atherosclerosis Index (A.I), Cardiac Risk Ratio (C.R.R) and Atherogenic Coefficient (A.C) in hyperlipidemic rats

Treatment Groups	A.I	C.R.R.	A.C
Vehicle Control	1.41±0.04	2.89±0.12	1.89±0.12
Disease Control	8.38±0.42	12.01±0.70	11.01±0.70
Standard Control	1.54±0.05** (81.62)	3.02±0.06** (74.85)	2.02±0.06** (81.65)
MEBB	2.52±0.19** (69.93)	4.21±0.23** (64.95)	3.21±0.23** (70.84)
EESC	2.56±0.07** (69.45)	4.35±0.10** (63.78)	3.35±0.10** (69.57)

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett's test (p**<0.01) Values in parenthesis indicate % decrease in respective parameters.

Determination of hepatic lipids

The groups treated with MEBB, EESC and standard drug Atorvastatin demonstrated a significant ($p<0.01$) decrease in Hepatic total cholesterol and Hepatic triglycerides when compared with disease control group. Hepatic total cholesterol was decreased by 53.12%, 51.34% and 63.99% by MEBB, EESC and Atorvastatin respectively. Hepatic triglycerides was decreased by 67.72%, 56.50% and 77.15% by MEBB, EESC and Atorvastatin respectively (Table 3)

Antioxidant activity in liver tissue homogenate

The groups treated with MEBB, EESC and standard drug Atorvastatin demonstrated a significant ($p<0.01$) inhibition of lipid peroxidation in liver when compared with disease control group (Table 4). There was significant ($p<0.01$) elevation in levels of reduced glutathione and Catalase in liver in groups treated with MEBB, EESC and standard drug Atorvastatin when compared with disease control group (Table 4).

Table 3: Effect of MEBB and EESC on Hepatic Total Cholesterol and Hepatic Triglyceride levels in hyperlipidemic rats

Treatment Groups	Hepatic Cholesterol (mg/g)	Hepatic Triglycerides (mg/g)
Vehicle Control	2.33± 0.14	6.86± 0.12
Disease Control	7.94± 0.68	19.16± 0.12
Standard Control	4.35± 0.13** (63.99)	9.67± 0.15** (77.15)
MEBB	4.96± 0.05** (53.12)	10.83±0.12** (67.72)
EESC	5.06± 0.07** (51.34)	12.21±0.23** (56.50)

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett's test (p**<0.01) Values in parenthesis indicate % decrease in respective parameters.

Table 4: Effect of MEBB and EESC on Lipid peroxidation, reduced glutathione and Catalase levels in livers of hyperlipidemic rats

Treatment Groups	Lipid Peroxidation (µMol/g wt. of wet liver tissue)	Reduced Glutathione (µMol/g wt. of wet liver tissue)	Catalase (µMol/g wt. of wet liver tissue)
Vehicle Control	9.26±0.012	2.37±0.054	67.95±0.40
Disease Control	18.92±0.0006	1.93±0.005	41.79±0.34
Standard Control	10.83±0.012 (83.75) ↓	2.2±0.009 (61.36)↑	62.95±0.40 (80.89)↑
MEBB	13.68±0.02 (54.24)↓	2.15±0.005 (50.00)↑	58.17±0.35 (62.61) ↑
EESC	13.19±0.02 (59.32) ↑	2.12±0.01 (43.18) ↑	58.01±0.33 (62.00) ↑

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett's test (p*<0.05, p**<0.01, p***<0.001) Values in parenthesis indicate % increase or decrease in respective parameters, ↓ indicates decrease; ↑ indicates increase.

DISCUSSION

The aim of the present study was to determine the antihyperlipidemic effect of *Bambusa bambos* and *Swertia chirata* in Cholesterol suspension induced hypercholesterolemia in rats. Administration of dietary cholesterol along with saturated fats results in accumulation of intracellular cholesterol and its esters in body tissues. Coconut oil contains approximately 92% saturated fats i.e. saturated fatty acids of short chain 15%, medium chain 64.2% and long chain 12.2% [21]. Therefore in present study, cholesterol suspension was formulated in coconut oil for purpose of dosing. High intake of dietary cholesterol increases serum lipid profiles by down regulation of LDL-C receptor synthesis thus decreasing uptake of LDL-C via these receptors [22].

MEBB and EESC showed significant ($p<0.01$) decrease in serum levels of Total cholesterol, triglycerides, LDL-C, VLDL-C and a concomitant increase in serum HDL-C levels. Reduction in serum total cholesterol levels may be due to inhibition of intestinal absorption of cholesterol [23], increase in expression of hepatic LDL-C receptors which accelerates the removal of LDL-C from blood and enhances degradation of cholesterol from body [24], inhibition of rate limiting enzyme in cholesterol synthesis i.e. 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase)[24] or activation of Cholesterol 7α-hydroxylase which catalyses the synthesis of bile acids from cholesterol termed as "classic pathway" thus enhancing clearance of cholesterol from body tissues [25].Lowered serum total cholesterol and LDL-C levels might be

useful in reducing the risk of coronary heart disease [26]. Serum HDL-C level may be elevated due to increased activity of lecithin cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL-C. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to liver for its catabolism (reverse cholesterol transport), thereby enhancing the clearance of cholesterol from body [27]. Therefore, increased serum HDL-C levels may prove beneficial in lipid disorders, have a protective role in Coronary artery disease [28] and may also act as cardio protective factor to prevent the gradual initiation of atherosclerotic process [29,30].

Oxidative stress is one of the causative factors that link hyperlipidemia with the pathogenesis of atherosclerosis. This stress results from an imbalance between production of free radicals and effectiveness of antioxidant defense system [31]. The main targets for the free radicals are polyunsaturated fatty acids in membrane of lipids causing lipid peroxidation which may lead to disorganisation of cell structure and function [32]. Lipid peroxidation, a free radical mediated process has been accepted to be one of the primary causes in development of cholesterol induced diseases. Malondialdehyde (MDA), a stable secondary product of lipid peroxidation cascade is widely used as a marker for measurement of lipid peroxidation [33]. Reduced glutathione and Catalase play unique role in cellular defense system by their ability to scavenge free radicals, as such depletion of reduced glutathione and catalase increases vulnerability to free radicals [34, 35]. Treatment with MEBB and EESC significantly ($p<0.01$) decreased hepatic MDA levels while increasing hepatic reduced glutathione and catalase levels. Administration of MEBB and EESC showed significant ($p<0.01$) antioxidant activity in liver tissue homogenate and may have protective role against oxidative stress induced atherosclerosis.

Atherogenic indices are powerful indicators of the risk of development of heart disease: higher the value, higher the risk of development of cardiovascular disease and vice versa [36, 37]. Treatment with MEBB and EESC lowered atherogenic indices significantly ($p<0.01$) thus decreasing the risk of development of cardiovascular disease.

Our results suggest that MEBB and EESC have effective hypocholesterolemic action together with antioxidant effect. Detailed studies are needed to postulate the possible mechanism(s) of action.

REFERENCES

1. Dorland. Dorland's Illustrated Medical Dictionary. 32nd ed. Philadelphia: Saunders; 2011.
2. Brunton LL, Chabner B, Knollman B Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill Medical; 2011. p. 603-20.
3. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2008.
4. Gogate VM. 2000. Ayurvedic pharmacology and therapeutic uses of medicinal plants (Dravyagunavignyan). Mumbai: Bharatiya Vidya Bhavan; 2008. p.717.
5. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Heidelberg: Springer; 2007. p.92.
6. Joshi SG. Medicinal Plants. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd; 2004. p. 314.
7. Miura T, Fukuta H, Ichiki H, Iwamoto N, Kubo M, Komatsu Y et al Hypolipidemic activity of Mangiferin in cholesterol-fed mice. J Trad Med 2001;18: 119–21.
8. Vaidya H, Rajani M, Sudarsanam V, Padh H, Goyal R Antihyperlipidemic activity of swertiamarin, a secoiridoid glycoside in poloxamer-407-induced hyperlipidemic rats. J Nat Med 2009;63: 437–42.
9. Dhuley J, Naik SR, Rele S,Banerji A Hypolipidemic and antioxidant activity of diallyl sulphide in rats. Pharm Pharmacol Commun 1999;5: 689-96.
10. Folch J, Lees M, Sloane Stanle GH A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226: 497–509.
11. Gopal SR, Raymond JL Automated enzymatic measurement of total cholesterol in serum. Clin. Chem. 1978;24: 108-14.
12. Michael WM, Joseph DA, Donald RS, Bennie ZA A Peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem 1983;29: 538-42.
13. Burstein M, Scholnick HR, Morfin R Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970;11: 583-95.
14. Friedewald WT, Levy RI, Fredrickson DS Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem 1972;18: 499–502.
15. Mertz DP "Atherosclerosis-index" (LDL/HDL): risk indicator in lipid metabolism disorders. Med. Klin 1980;75: 159–61.
16. Malaspina JP, Bussière H, Le Calve G The total cholesterol/HDL cholesterol ratio: a suitable atherogenesis index. Atherosclerosis 1981;40: 373–75.
17. Kayamori F, Igarashi K Effects of dietary nasunin on the serum cholesterol level in rats. Biosci Biotechnol Biochem 1994;58: 570–71.
18. Buege JA, Aust SD Microsomal lipid peroxidation. Methods. Enzymol 1978;2: 302–10.
19. Anderson ME Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113: 548–55.
20. Aebi H Catalase in vitro. Methods Enzymol 1984;105: 121–26.
21. Ochani PC, D'Mello P Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. leaves and calyces extracts in rats. Indian J Exp Biol 2009;47: 276-82.
22. Dietschy JM, Turley SD, Spady DK Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 1993; 34: 1637–59.
23. Van HM, Compton DS, Davis HR The cholesterol absorption inhibitor, ezetimibe, decreases diet-induced hypercholesterolemia in monkeys. Eur J Pharmacol 2001;415: 79–84.
24. Brown MS, Goldstein JL A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232: 34–47.
25. Myant NB, Mitropoulos KA Cholesterol 7 alpha-hydroxylase. J Lipid Res 1977;18: 135–53.
26. Tamir I, Heiss G, Glueck CJ, Christensen B, Kwiterovich P, Rifkind BM Lipid and lipoprotein distributions in white children ages 6–19 yr. The Lipid Research Clinics Program Prevalence Study J Chronic. Dis 1981;34: 27–39.
27. Bandeali S, Farmer J High-density lipoprotein and atherosclerosis: The role of antioxidant activity. Curr Atheroscler 2012;14: 101–7.
28. Khanna, AK, Chander R, Singh C, Srivastava AK, Kapoor NK Hypolipidemic activity of *Achyranthus aspera* Linn in normal and triton induced hyperlipemic rats. Indian J Exp Biol 1992;30: 128–30.
29. Barter P The role of HDL-cholesterol in preventing atherosclerotic disease. Eur Heart J Suppl 2005;7: F4–F8.
30. Chapman, MJ Therapeutic elevation of HDL-cholesterol to prevent atherosclerosis and coronary heart disease. Pharmacol Ther 2006;1 11:893–908.
31. Halliwell B Mechanisms involved in the generation of free radicals. Pathol Boil 1996;44: 6–13.
32. Rahman K Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007;2: 219–36.
33. Tiwari AK Natural product antioxidants and their therapeutic potential in mitigating peroxidative modification of lipoproteins and atherosclerosis: recent development. J Med Aroma Plant Sci 1999;21: 730-41
34. Reed DJ Glutathione: Toxicological Implications. Annu Rev Pharmacol Toxicol 1990;30: 603–31.
35. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O Oxidants and antioxidants in atherogenesis. An appraisal. J Lipid Res 1999;40: 2143–57.
36. Frohlich J, Dobiásová M Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography. Clin Chem 2003;49: 1873–80.
37. Martirosyan, DM, Miroshnichenko LA, Kulakova SN, Pogojeva AV, Zoloedov VI Amaranth oil application for coronary heart disease and hypertension. Lipids Health Dis 2007; 6: 1.