



Research Article

ANTI-HYPERLIPIDEMIC EFFECT OF TRIGLIZE, A POLYHERBAL FORMULATION

PARASURAMAN S^{1*}, KUMAR EP¹, ANIL KUMAR², EMERSON SF³

¹Department of Pharmacology, J.S.S College of Pharmacy, Ootacamund, India, ²TIFAC-CORE, J.S.S College of Pharmacy, Ootacamund, India, ³Green Milk Concepts, Herbal Division of Apex Laboratories, Chennai 600038, India. Email: parasuphd@gmail.com

Received: 15 Feb 2010, Revised and Accepted: 16 March 2010

ABSTRACT

Anti-hyperlipidemic property of Trigize, a polyherbal formulation was determined in male albino Wistar rats. The aqueous extract of polyherbal formulation (PHF) trigize was used for the study. The animals were fed with high fat diet to induce hyperlipidemia. The test (polyherbal formulation) and standard drugs were administered once daily as a single oral dose for 28 days. The test drug administered at the dose levels of 25, 50, 100 and 200 mg/kg and standard drug atorvastatin was administered at the dose level of 10 mg/kg. Weekly body weight variation, lipid profile (total cholesterol, triglyceride, HDL, VLDL and LDL levels, HDL ratio and atherogenic index) were analyzed and day of the termination the liver was isolated from the all the animals and subjected to histopathological evaluation. End of the experiments liver markers like ASAT, ALAT were analyzed in serum samples. High fat diet showed significant increase in body weight, lipid profile in serum. The polyherbal formulation and atorvastatin treated groups showed significantly increased HDL levels and decreased total cholesterol, triglyceride, LDL, VLDL, HDL ratio and atherogenic index. End of the study atorvastatin elevated the ASAT and ALAT levels. The PHF has been found significant anti-hyperlipidemic and hepatoprotective action.

Keywords: Polyherbal formulation, High fat diet, Anti-hyperlipidemia

INTRODUCTION

Ischemic heart disease (IHD) which includes angina pectoris, myocardial infarction, chronic postischemic cardiac failure and sudden ischemic death is most common and leading causes of morbidity and mortality in developed and developing countries.¹ Hyperlipidemia, hypertension, obesity, raised coagulation factor and homocysteine are modified risk factors for atherosclerosis. In that dyslipidemia is most common risk factor causes IHD in elderly population. The underlying mechanism of IHD involves the deposition of serum lipids in coronary arteries, and its resulting in decreased blood flow to cardiac muscles. Based on the hypothesis dyslipidemia is treated with cholesterol biosynthesis reducing agents like statins (3-hydroxy-3-methylglutaryl coenzyme A reductase), fibrates (activates peroxisome proliferator-activated receptor α and it stimulates β -oxidative degradation of fatty acids) etc.² Statins have been found to be more effective (21% to 43%) against serum low-density lipoprotein level and they cause many side effects like liver function elevations, gastrointestinal disturbance, insomnia and myalgia.^{2,3} Ayurveda is one of the Indian systems of medicine and that has been practiced in India for more than 2000 years.⁴ The marketed polyherbal formulation (ayurvedic formulation) Trigize claims that it can be used for the treatment of obesity, hypertension, palpitation, myocardial necrosis, coronary atherosclerosis, hypercholesterolemia, ischemic heart diseases and peripheral vascular diseases. Dyslipidemia is playing a major role in ischemic heart diseases, so the present study was planned to determine the anti-hyperlipidemic effect of Trigize, a polyherbal formulation (PHF) in high fat diet induced hyperlipidemia in an animal model.

MATERIALS AND METHODS

Polyherbal formulation (PHF)

Trigize (Batch No.: 13335002, Mfg date: Jun, 2005, Apex Laboratories Ltd., Chennai) is a marketed soft gelatin capsule and it was formulated using aqueous extracts of *Terminalia arjuna*, *Cissus quadrangularis*, *Boerhaavia diffusa*, *Commiphora mukul*, *Phyllanthus emblica*, *Terminalia belletica*, *Terminalia chebula*, *Tribulus terrestris*, *Allium sativum* and *Trigonella foenum-graecum*.

Animals

Healthy, adult, male albino Wistar rats, weighing 150-175 g were obtained from J.S.S. College of Pharmacy, Animal House, Ootacamund, Tamil Nadu, India. The rats were housed under 22 \pm 2 $^{\circ}$ C temperature, 40-60 % humidity and 12-12 \pm 1 h light dark cycle. The animals were housed in large, spacious, hygienic cages during the course of experimental period. During course of the experimental period, the animals were fed freshly prepared with high fat diet (HF) (15 g/ animal) throughout the experimental period except normal untreated group (group-1). Group 1 animals were fed with freshly prepared normal diet. The experimental animals were allowed free access with drinking water. The study was approved by the Institute Animal Ethics Committee and all the animal experiments were carried out as per CPCSEA guidelines.

Composition of normal and high fat diet

Composition of normal fed was 27 % whole wheat, 25 % yellow corn, 15 % barley, 15 % milk powder, 1 % bone meal, 1 % calcium chloride, 1 % sodium chloride 15 % coconut oil and one multivitamin capsule. The high fat diet was contained 23 % whole wheat, 23 % yellow corn, 11 % barley, 17 % milk powder, 1 % bone meal, 1 % calcium chloride, 1 % sodium chloride 11 % coconut oil, 11 % butter and one multivitamin capsule.

Anti-hyperlipidemic effect of PHF

Forty two male Wistar rats (150-175 g) were divided into six groups containing six animals and grouped as follows.

Group 1 (normal diet): Normal diet (15 g/day/rat) + Drug vehicle (1 ml/kg)

Group 2 (HFD control): HF diet (15 g/day/rat) + Drug vehicle (1 ml/kg)

Group 3: HF diet (15g/day/rat) + Atorvastatin (10 mg/kg)

Group 4: HF diet (15g/day/rat) + PHF (25 mg/kg)

Group 5: HF diet (15g/day/rat) + PHF (50 mg/kg)

Group 6: HF diet (15g/day/rat) + PHF (100 mg/kg)

Group 7: HF diet (15g/day/rat) + PHF (200 mg/kg)

The PHF and standard drug atorvastatin were administered once daily as a single oral suspension for 28 days. The oral suspension was prepared with 0.5 % w/v carboxymethyl cellulose (CMC). All the experimental procedure was carried out between 7:30 am to 10:00 am. The standard and test substance were administered between 8:00 am to 9:00 am. Throughout the experimental period weekly body weights variation of experimental animals were recorded for all the groups. End of the experiment the animals were euthanized with high dose of ketamine i.p. injection and gross pathology of animals were examined.

Biochemical estimations

One ml of blood sample was collected weekly for four weeks from the eight hours fasted animals, from all the groups through retro orbital puncture under mild ether anaesthesia. The blood samples were collected in plain glass tubes and allowed to clot for 20 minute at room temperature and centrifuged at 3000 RPM for 20 minute. The serum obtained was kept at 0 °C until analyzed. Serum was used for the estimation of the serum lipid profile like total cholesterol, triglyceride, HDL-cholesterol, liver enzyme parameters like Aspartate aminotransferase (ASAT), Alanine amino transferase (ALAT) and glucose by using Microlab 100 semi auto-analyzer (Vital Scientific N.V., The Netherland). The lipid profile, liver enzyme profile, glucose were estimated using Randox, E-merk India Ltd., and Lab kit enzymatic kits respectively. The LDL-cholesterol, VLDL-cholesterol, HDL ratio and atherogenic index (AI) were calculated mathematically.⁵⁻⁷

Histopathological Study

End of the experiment, the liver, kidney, heart and peritoneal fats were excised and absolute organ weight was measured. To find out the influence of PHF on histology of liver, the histopathology of liver was carried out. The excised liver was fixed in 10% v/v buffered neutral formalin. Part of the liver sample was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five micrometer thickness of liver section were prepared from paraffin blocks and stained with hematoxylin and eosin and mounted in neutral DPX medium,⁸ and the sections were examined under light microscope.

Statistical Analysis

The values were expressed as mean ± SEM for each group. Significant difference between groups was determined using one-way ANOVA followed by Tukey's multiple comparison test. A P value less than 0.05 was considered significant.

RESULTS

Body weight analysis

Effect of HFD, PHF and atorvastatin on animals body weight were analyzed on 0, 7th, 14th, 21st and 28th day of the experiment. The HFD fed animals showed significant increases in body weight compared to normal diet fed animals from second week onwards (p<0.001). The standard drug treated group, 25, 50, 100 and 200 mg/kg PHF treated groups not showed any significant increasing in body weight compared with normal diet fed animals throughout the experimental period.

Serum lipid profile

The effect of PHF on lipid profile (total cholesterol, triglyceride, HDL, VLDL and LDL levels, HDL ratio and AI) over a period of 28 days treatment, in the treatment and control groups were analysed on 7th, 14th, 21st, 28th day of treatment and the values were depicted in the tables (Tables 1,2,3 and 4).

In HFD fed rats showed significant percentage increase in lipid profile level from the first week of the experiment onwards. The percentage changes in total cholesterol levels in 50, 100 and 200 mg/kg treated groups on 15th day of the study was 34.67% (107.17±12.6 to 144.33±5.94 mg/dl); -6.89% (94.33±7.03 to 87.83±4.33 mg/dl) and -11.11% (99.0±6.09 to 110.0±4.86 mg/dl) respectively. On the day of termination the PHF treatment groups showed the significant percentage changes in total cholesterol at the dose levels of 50, 100 and 200mg/kg b.wt was 4.83% (107.17±12.6 to 112.17±6.68 mg/dl); 10.43% (94.33±7.03 to 104.17±6.0 mg/dl) and -7.07% (99.0±6.09 to 92.0±4.57 mg/dl) respectively.

The percentage changes in triglyceride levels in 50, 100 and 200 mg/kg treated groups on 15th day of the study was -51.43% (120.83±5.9 to 58.67±5.67 mg/dl); -7.46% (56.17±2.96 to 52.0±4.16 mg/dl) and -46.34% (58.83 to 65.33±10.31 mg/dl) respectively. On the day of termination the PHF treatment groups showed the significant percentage changes in triglyceride at the dose levels of 50, 100 and 200mg/kg b.wt -46.34% (120.83±5.9 to 64.83±5.48 mg/dl); 3.84% (56.17±2.96 to 58.33±8.63 mg/dl) and 3.12% (58.83 to 60.67±4.30 mg/dl) respectively.

Significant increase in HDL level was found in 25, 50mg/kg PHF treated group on second and third week. Significant reduction of calculated VLDL, AI found in both standard and PHF treatment groups on 2nd, 3rd and 4th weeks of treatment.

Table 1: Effect of polyherbal formulation on lipid profile in serum- (Week I) on HFD fed Wistar rats

S. No	Treatment	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Ratio	Atherogenic Index
1	Control	83.17±4.33	84.50±6.91	26.5±1.18	39.77±4.36	16.9±1.38	3.17±0.22	2.22±6.52
2	HFD control	143.67±4.24 [#]	175.17±7.2 [#]	24.0±1.48	84.63±4.17 [#]	35.03±1.44 [#]	6.08±0.34 [#]	6.52±0.35 [#]
3	Atorvastatin	97.67±10.32 ^{***}	72.17±7.75 ^{***}	26.17±1.64	57.07±6.90	14.43±1.55 ^{***}	3.73±0.23 ^{***}	1.83±0.35 ^{***}
4	PHF	128.67±10.32	75.50±5.89 ^{***}	30.33±1.61	83.23±11.85	15.10±1.18 ^{***}	4.35±0.49 ^{**}	1.51±0.21 ^{***}
5	PHF 25mg/kg	107.17±12.6	120.83±5.9 ^{***}	26.50±0.85	56.50±7.44	24.17±1.17 ^{***}	4.07±0.28 ^{***}	3.57±0.23 ^{***}
6	PHF 50mg/kg	94.33±7.03 [*]	56.17±2.96 ^{***}	32.67±2.44 ^{**}	50.43±7.04 [*]	11.23±0.59 ^{***}	2.96±0.27 ^{***}	0.76±0.23 ^{***}
7	PHF 100mg/kg	99.0±6.09 ^{**}	58.83±5.64 ^{***}	28.83±1.49	58.40±5.71	11.77±1.13 ^{***}	3.46±0.20 ^{***}	1.04±0.16 ^{***}
	PHF 200mg/kg							

(Values are mean ± SEM of six animals). [#]p<0.01, ^{*}p<0.001 as compared to Control; ^{*}p<0.05, ^{**}p<0.01, ^{***}p<0.001 as compared to HFD. One way ANOVA by Tukey's multiple comparison test.

Table 2: Effect of Polyherbal formulation on lipid profile in serum- (Week II) on HFD fed Wistar rats

S. No	Treatment	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Ratio	Atherogenic Index
1	Control	103.0±5.35	50.17±1.90	27.83±1.35	65.13±5.41	10.03±0.38	3.62±0.17	0.82±0.09
2	HFD control	176.17±10.04 [#]	168.0±8.47 [#]	26.67±1.69	115.90±9.77 [*]	33.60±1.69 [#]	6.72±0.31 [#]	5.38±0.37 [#]
3	Atorvastatin	124.83±9.52	72.83±6.26 ^{***}	27.83±1.28	82.43±9.86	14.57±1.25 ^{***}	4.98±0.60	1.64±0.25 ^{***}
4	PHF 25mg/kg	130.50±11.23 ^{**}	63.67±3.32 ^{***}	36.67±1.28 ^{**}	81.10±11.30	12.73±11.73 ^{***}	4.51±0.86 ^{***}	0.75±0.11 ^{***}
5	PHF 50mg/kg	144.33±5.94	58.67±5.67 ^{***}	30.33±1.38	102.27±6.88	11.73±1.13 ^{***}	4.82±0.37	0.95±0.20 ^{***}
6	PHF 100mg/kg	87.83±4.33 ^{***}	52.0±4.16 ^{***}	34.0±2.07	43.42±6.39 ^{***}	10.40±0.83 ^{***}	2.34±0.08 ^{***}	0.56±0.15 ^{***}
7	PHF 200mg/kg	110.0±4.86 ^{***}	65.33±10.31 ^{***}	39.83±2.89	55.40±7.28 ^{***}	13.07±2.06 ^{***}	2.82±0.31 ^{***}	0.69±0.29 ^{***}

(Values are mean ± SEM of six animals). ^{*}p<0.01, [#] p<0.001 as compared to Control; ^{*}p<0.05, ^{**}p<0.01, ^{***}p<0.001 as compared to HFD. One way ANOVA by Tukey's multiple comparison test.

Table 3: Effect of Polyherbal formulation on lipid profile in serum- (Week III) on HFD fed Wistar rats

S. No	Treatment	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Ratio	Atherogenic Index
1	Control	102.17±10.16	74.67±6.80	27.67±1.28	59.57±6.73	14.93±1.36	3.73±0.30	1.78±0.36
2	HFD control	218±10.16 [#]	115.0±7.48 [#]	27.33±1.54	167.67±9.91 [#]	23.0±1.5 [§]	8.09±0.60 [#]	3.32±0.44 [§]
3	Atorvastatin	140.0±12.11 ^{***}	73.17±8.63 ^{**}	39.33±2.93 ^{**}	86.03±14.51 ^{***}	14.63±1.73 [*]	3.71±0.51 ^{***}	0.88±0.21 ^{***}
4	PHF 25mg/kg	137.17±9.90 ^{***}	62.60±6.05 ^{***}	35.17±1.68	91.57±10.79 ^{***}	10.43±2.35 ^{***}	3.90±0.22 ^{***}	0.58±0.33 ^{***}
5	PHF 50mg/kg	92.50±6.30	77.50±8.61 ^{***}	40.50±2.79 ^{***}	36.50±5.44 ^{***}	15.50±1.72 [*]	2.33±0.21 ^{***}	0.92±0.17 ^{***}
6	PHF 100mg/kg	84.50±4.34 ^{***}	67.67±2.11 ^{***}	35.17±1.70	36.80±3.98 ^{***}	13.53±0.42 ^{**}	2.42±0.14 ^{***}	0.95±0.11 ^{***}
7	PHF 200mg/kg	97.83±4.35 ^{***}	52.83±6.25 ^{***}	37.83±1.49	49.43±5.50 ^{***}	10.57±1.25 ^{***}	2.61±0.15 ^{***}	0.40±0.15 ^{***}

(Values are mean ± SEM of six animals). [§] p<0.05, [§]p<0.01, [#] p<0.001 as compared to Control; ^{*}p<0.05, ^{**}p<0.01, ^{***}p<0.001 Vs HFD. One way ANOVA by Tukey's multiple comparison test.

Table 4: Effect of Polyherbal formulation on lipid profile in serum- (Week IV) on HFD fed Wistar rats

S. No	Treatment	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Ratio	Atherogenic Index
1	Control	101.50±7.68	54.0±3.04	26.33±2.73	64.37±9.07	10.80±0.61	4.09±0.58	1.16±0.25
2	HFD control	148.33±7.25 [#]	118.0±16.11	29.50±1.61	95.23±9.94 [#]	23.60±3.22 [#]	5.14±0.48	3.04±0.54 [#]
3	Atorvastatin	111.2±3.18	59.50±4.37 ^{***}	36.50±1.73	62.93±4.44	11.90±0.87 ^{***}	3.10±0.22 ^{**}	0.66±0.16 ^{***}
4	PHF 25mg/kg	128.17±5.10	47.67±5.48 ^{***}	37.17±1.90	81.47±5.67	9.53±1.10 ^{***}	3.15±0.28 [*]	0.60±0.15 ^{***}
5	PHF 50mg/kg	112.17±6.68 ^{**}	64.83±5.48 ^{***}	38.17±2.88	61.03±5.65 [*]	12.97±1.11 ^{***}	2.99±0.21 ^{**}	0.79±0.27 ^{***}
6	PHF 100mg/kg	104.17±6.0 ^{***}	58.33±8.63 ^{***}	34.67±1.33	57.83±5.65 ^{**}	11.67±1.73 ^{***}	3.02±0.18 ^{**}	0.65±0.17 ^{***}
7	PHF 200mg/kg	92.0±4.57 ^{***}	60.67±4.30 ^{***}	37.33±3.26	45.53±6.50 ^{***}	12.13±0.86 ^{***}	2.85±0.38 ^{**}	0.88±0.27 ^{***}

(Values are mean ± SEM of six animals). ^{*}p<0.01, [#] p<0.001 as compared to Control; ^{*}p<0.05, ^{**}p<0.01; ^{***}p<0.001 as compared to HFD. One way ANOVA by Tukey's multiple comparison test.

Table: 5 Effect of Polyherbal Formulation on relative organ weight analysis on HFD fed Wistar rats

S. No	Treatment	Relative organ weight (gms)			
		Heart	Liver	Kidney	Peritoneal fat
1	Control	0.364±0.021	2.487±0.35	0.663±0.031	0.644±0.049
2	High fat diet control	0.317±0.025	2.640±0.18	0.575±0.026	1.275±0.12 ^{***}
3	Atorvastatin	0.367±0.014	3.763±0.028 ^{**}	0.805±0.105 [*]	0.814±0.19 ^{**}
4	PHF 25mg/kg	0.375±0.02	3.118±0.18 ^{**}	0.659±0.15	0.684±0.62
5	PHF 50mg/kg	0.355±0.018	2.994±0.031 [*]	0.746±0.066	0.653±0.12
6	PHF 100mg/kg	0.349±0.021	3.152±0.209 ^{**}	0.672±0.028	0.667±0.19
7	PHF 200mg/kg	0.322±0.024	2.931±0.194	0.647±0.032	0.825±0.096 ^{**}

(Values are mean ± SEM of six animals). p value ^{***}<0.001, ^{**}<0.01, ^{*}<0.05 as compared to control.

Serum liver enzymes

Effect of PHF and atorvastatin on liver function (ALAT, ASAT, total protein and glucose) was assessed on pre-study day and study termination day using serum sample. End of the experiment 25, 50, 100 and 200 mg/Kg PHF treated groups not showed significant increase in ALAT, ASAT, total protein and glucose levels, but 10 mg/kg atorvastatin treated groups showed significant increase in ALAT, ASAT levels on end of the study ($p < 0.01$).

Necropsy and organ weight analysis

The gross pathological examinations not showed abnormality attributed by the treatment with PHF at four dose level 25, 50, 100 and 200 mg/kg b.wt. The absolute organ weight of the isolated liver, heart, kidney and peritoneal fat from all the groups were recorded and relative organs weight were calculated (Table 5). Atorvastatin 10 mg/kg shows significantly increase in liver and kidney weight ($p < 0.01$). The PHF at the dose levels of 25, 50, 100 mg/kg does not showed any significant increase in liver weight. The animals were fed with HFD diet and treat with atorvastatin showed significant increased peritoneal fat content ($p < 0.001$; $p < 0.01$). The liver histopathological examination not showed marked significant changes. The HFD fed animals showed fatty infiltration and minimal periportal inflammation.

DISCUSSION

The present study result supports the hypolipidemic property of a polyherbal formulation. Findings of lipid profile of PHF treatment in all the doses showed a significant decrease in triglyceride, total cholesterol, VLDL, LDL, significant increase in HDL values at 25 mg/kg and 50 mg/kg and significant increase in HDL:Tc ratio at the all the dose levels, when compared with control group over a period of 28 days treatment. Here the composition of high fat diet is changed because of cassine causes allergic reactions in animal's skin, so the composition of the high fat diet was changed.

In present study, the high fat diet contains more amounts of natural substance includes butter and coconut oil. Butter is a rich source of saturated fats and its contains about 81.11 % of total lipid, 51.37 % of total saturated fatty acids and 21.02 % of total monounsaturated fatty acids and its helps to increase cholesterol levels in this model. (National agriculture library, United States dept of agriculture)

The PHF shows significant increase in HDL level and decreases in LDL, VLDL levels. Increase in LDL, VLDL levels are increase the risk of cardiovascular diseases. Increases of HDL have cardioprotective effect and it was proved by various studies.^{9,10}

Atherogenic index (AI) is used as a marker to assess the susceptibility of atherogenesis.¹¹ Significant reduction of AI was found in PHF treated group and the study results indicating protective role of PHF against atherogenesis.

The PHF formulated by using more than 10 individual plants. The hypolipidemic properties of *Commiphora mukul*, *Emblica officinalis*, *Zingiber officinale*, *Tribulus terrestris* and *Semecarpus anacardium* were reported. The antioxidant property of *Terminalia arjuna*, *Emblica officinalis* and *Aegle marmelos* were also reported.¹²⁻¹⁷

The currently used hyperlipidemic drugs lag behind the desired properties such as efficacy and safety on long term use, cost and simplicity of administration. These factors fulfill conditions for patient complication. Herbs are mines of medicinal agents and needs for researchers are felt to find efficacious, cheap and safe hyperlipidemic agents from among the natural products.¹⁸

CONCLUSION

The present study conclude that, the "Triglize" a polyherbal formulation has anti-hyperlipidemic property against high fat diet induced hyperlipidemia in rats and the polyherbal formulation not showing any liver enzyme abnormalities.

REFERENCES

1. Carnethon MR, Lynch EB, Dyer AR, Lloyd-Jones DM, Renwei W, Garside DB, Greenland P. Comparison of risk factors for cardiovascular mortality in black and white adults. *Arch Intern Med* 2006; 166:1196-1202.
2. Rang HP, Dale MM, Ritter JM, Moore PH. *Pharmacology*. 5th ed. Churchill livingstone, Edinburgh.
3. Silva MA, Swanson AC, Gandhi PJ, Tataronis GR. Statin-related adverse events: a meta-analysis. *Clin Ther* 2006; 28:26-35.
4. Singh BB, Vinjamury SP, Der-Martirosian C, Kubik E, Mishra LC, Shepard NP, et al. Ayurvedic and collateral herbal treatments for hyperlipidemia: a systematic review of randomized controlled trials and quasi-experimental designs. *Alternative Therapies* 2007; 13:22-8.
5. Freidwald 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.
6. Friedlander Y, Kark JD, Eisenberg S, Stein Y. Calculation of LDL-cholesterol from total cholesterol, triglyceride and HDL-cholesterol: a comparison of methods in the Jerusalem Lipid Research Clinic Prevalence Study. *Isr J Med Sci*. 1982; 18:1242-52.
7. Noriko S, Mitsuhide N, Takeshi U, Tetsuya T, Takayoshi S, Kazunori Y, et al. Leptin-to-Adiponectin Ratio as a Potential Atherogenic Index in Obese Type 2 Diabetic Patients. *Diabetes care* 2004; 27:2488-90
8. Mukherjee, KL. *Medical Laboratory Technology*, vol- 1. Tata MC-Graw Hill Publication Company, New Delhi; 1988.
9. Martin M, Annie B. The positive relationship between alcohol and heart disease in eastern Europe: potential physiological mechanisms. *J R Soc Med* 1998; 9:402-7.
10. Takaaki S, Vajjinth SK, Moti LK. Niacin, but not gemfibrozil, selectively increases LP-AI, a cardioprotective subfraction of HDL, in patients with low HDL cholesterol. *Arterioscler Thromb Vasc Biol* 2001; 21:1783-9.
11. Kottai MA, Suthupathy S, Manavalan R, Karar PK. Hypolipidemic effect of methenolic extract of *Dolichos biflorus* Linn. in high fat diet fed rats. *Indian J Exp Biol* 2005; 43:522-5.
12. Fawzia MR, Amr YE, Soad MA, Aida MI, Mona A. The antihyperlipidemic activities of 4(3H) quinazolinone and two halogenated derivatives in rats. *Lipids Health Dis* 2005; 4: 22.
13. Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. *J Med Food* 2004; 7:343-8.
14. Wang X, Greilberger J, Ledinski G, Kager G, Paigen B, Jurgens G. The hypolipidemic natural product *Commiphora mukul* and its component guggulsterone inhibit oxidative modification of LDL. *Atherosclerosis* 2004; 172:239-46.
15. Burris TP, Montrose C, Houck KA, Osborne HE, Bocchinfuso WP, Yaden BC, et al. The hypolipidemic natural product guggulsterone is a promiscuous steroid receptor ligand. *Mol Pharmacol* 2005; 67:948-54.
16. Anila L, Vijayalakshmi N R. Flavonoids from *Emblica officinalis* and *Mangifera indica*-effectiveness for dyslipidemia. *J Ethnopharmacol* 2002; 79:81-7.
17. Pawar RS, Bhutani KK. Effect of oleanane triterpenoids from *Terminalia arjuna*- a cardioprotective drug on the process of respiratory oxyburst. *Phytomedicine* 2005; 12:391-3.

18. Ram A. Effect of *Plumbago zeylanica* in hyperlipidemic rabbit and its modification by vitamin E. Indian J

Pharmacol 1996; 28:161-6.