ORIGINAL ARTICLE

Anti-hypercholesterolaemic activity of Lipovedic and its mechanism

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Objective: To illustrate the antihypercholesterolaemic mechanism of Lipovedic - a polyherbal formulation. **Materials and Methods:** The antihypercholesterolaemic mechanism of Lipovedic (180 mg/kg, p.o) was studied by measuring the serum total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), Faecal cholesterol (FC) and bile acid excretion in triton induced hyperlipidaemic rats at 0 h, 18 h, 24 h, 40 h, and 48 h and in high cholesterol diet (HCD) induced hypercholesterolaemic rats on day 7, 14, 21 and 28. Hydroxy-3-Methyl Glutaryl Co-Enzyme A (HMG CoA)/mevalonate ratio was measured in the liver homogenate for the HMG-CoA reductase activity. Atorvastatin (7.2 mg/kg p.o) was used as reference standard. One-way ANOVA, Post-hock analysis by Tukey's multiple comparison tests was done using GraphPad Prism 5. **Results:** Lipovedic (180 mg/kg, p.o) reduced the serum TC, TG, LDL-C, VLDL-C and increased the serum HDL-C in triton treated and HCD fed rats. Lipovedic increased FC excretion. HMG CoA/Mevalonate ratio was significantly higher compared to vehicle control rats and the result was comparable with Atorvastatin (7.2 mg/kg p.o). **Conclusion:** Lipovedic acts as an anti-hypercholesterolaemic drug by: inhibition of HMG CoA reductase, increasing FC excretion and reducing dietary cholesterol absorption, increasing hepatic low density lipoprotein receptor expression and by activation of Lecithin Cholesterol Acyl Transferase and Lipoprotein lipase.

Key words: High cholesterol diet, homogenate Co-Enzyme A reductase, hypercholesterolemia, Lipovedic, triton, carboxy methyl cellulose

INTRODUCTION

Hypercholesterolemia is characterized by elevated plasma total cholesterol (TC).[1] Epidemiologic studies have demonstrated a significant relationship between plasma cholesterol concentrations and coronary artery disease.[2] Major cause for the development of ischemic diseases is high cholesterol and related dyslipidaemias.[3] Therefore, treatment of hyperlipidaemia is a major approach towards decelerating atherogenic process. Allopathic hypolipidaemic drugs are available in the market, but the side effects and contraindications are the main drawbacks of these drugs. Herbal hypolipidaemics are gaining importance in recent times.[4] Ayurveda, an ancient science of medicine of Indian origin, lists many reliable, clinically useful, affordable herbal hypolipidaemics.

Lipovedic is a polyherbal formulation developed by Vedic Bio-labs, Bangalore containing *Commiphora mukul, Terminalia chebula, Terminalia belerica, Emblica officinalis, Piper longum, Piper nigrum, Zingeber officinalis, Mesua ferra, Plumbago zeylanica, Cyperus rotandus, Embelia ribes, Piper cubeba, Juniperus communis, Aconitum heterophyllum, Cissampelos pareira.* Lipovedic is intended in treatment of hypercholesterolemia and to reduce obesity. Earlier studies have confirmed that Lipovedic increases serum high density lipoprotein (HDL), glucose and significantly decreases cholesterol, low density lipoprotein (LDL), very low density lipoproteins (VLDL) and has anti-obesity activity.[5] This study is an attempt to screen the antihypercholesterolemic activity and elucidate the mechanism of action of the polyherbal formulation Lipovedic.

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MATERIALS AND METHODS

Drugs/Chemicals

Lipovedic was a gift sample by Vedic Bio-Labs Pvt Ltd, Bangalore. Triton WR 1339, Brij 30 was purchased from Sigma-Aldrich, U.S.A. Cholesterol, Triton X-100 and Sodium arsenate was purchased from SD-fine Chem Pvt., Ltd., India. Cholic acid was purchased from Hi Media Laboratories, India. Groundnut oil was purchased from the local market. Hydroxylamine hydrochloride was obtained from Fischer Scientific. Atorvastatin was obtained from Biocon Pvt, Ltd, India. Cholesterol and HDL estimation kit, Triglyceride (TG) estimation kit was obtained from Span diagnostics India (P) Ltd.

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Experimental Animals

Healthy, male albino rats, Wistar strain (175 ± 25 g) were procured from the Central Animal Research Facility, NIMHANS, Bangalore. The animals were maintained at 25°C ± 2°C and 55% ±10% relative humidity, with a natural light and dark cycle in the animal house of Department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. The rats were given standard pellet diet (Mysore Feeds, Nayandahalli, Bangalore) and provided with water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee before the start of the experiments (Reg. No. 152/1999/Committee for Purpose of Control and Supervision of Experiments on Animals, renewal on 12-10-2007).

Triton Induced Hyperlipidemia in Rats

Twenty four adult male rats weighing 150-200 g were divided into four groups of six animals each and were fasted for 18 h. Animals of first group served as vehicle control, received 0.2% w/v Carboxy Methyl Cellulose, 10 ml/kg p.o. The second group, hyperlipidaemic control rats, received Triton WR-1339, 200 mg/kg i.p,[6] in normal saline; the third and fourth group animals were treated with Atorvastatin, 7.2 mg/kg p.o.[7,8] and Lipovedic, 180 mg/kg p.o.,[5] respectively, for 7 consecutive days followed by Triton on 8th day. Thereafter, blood was collected at hour 0, 18, 24, 40 and 48 by retro-orbital puncture under light ether anaesthesia.[8] Serum was separated and used to estimate TC,[9] (TGs),[10] high density lipoprotein-cholesterol (HDL-C),[11] low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) levels.[12]

High Cholesterol Diet Induced Hypercholesterolaemic Rat Model

Twenty four adult male rats weighing 150-200 g were divided into four groups of six animals each. Animals of the first group served as vehicle control, received 0.2% w/v CMC, 10 ml/kg p.o. The second, hyperlipidaemic control rats, received HCD p.o. (cholesterol (500 mg/kg) + Cholic acid (50 mg/kg) suspended in Groundnut oil (0 ml/kg)), for 28 days consecutively; third and fourth group animals, received Atorvastatin, 7.2 mg/kg p.o., and Lipovedic, 180 mg/kg p.o. respectively, 1 h prior to administration of HCD for 28 consecutive days. Blood was collected on day 7, 14, 21 and 28 of the experiment by retro-orbital puncture under light ether anaesthesia.[8,13] The serum was analysed for TC,[9] TG,[10] HDL-C,[11] LDL-C, and VLDL-C.[12] Faeces was collected on days 7, 14, 21 and 28 of the experiment and analysed for faecal cholesterol (FC)[14] and faecal bile acids-cholic acid and desoxycholic acid.[15]

Serum cholesterol was estimated by the colorimetric, end point Cholesterol oxidase and peroxidase (CHOD-PAP) method, by adding 10 µl of the serum sample to 1000 µl of the reagent 1(cholesterol reagent), mixed well and incubated at room temperature for 10 min. To 1000 µl of the reagent 1(cholesterol reagent), 10 µl of standard cholesterol (200 mg/dl) was added and incubated for 10 min at room temperature. The absorbance was read at 505 nm using a semi auto analyser. Concentration of serum cholesterol (mg/dl) was calculated using formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Serum TG was estimated by the Enzymatic, end point, colorimetric, Glycerol-3-phosphate oxidase-peroxidase method using the TG kit. Serum sample, 10 µl was added to 1000 µl of the reagent 1 (TG mono reagent), mixed well and incubated at room temperature for 10 min. To 1000 µl of the reagent 1 (TG mono reagent), 10 µl of TG standard (200 mg/dl) was added and incubated for 10 min at room temperature. The absorbance was read at 505 nm using a semi auto analyser. Concentration of serum TG (mg/dl) was calculated using formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

HDL-C was estimated by Poly Ethylene glycol-CHOD-PAP, end point assay with Lipid Clearing Factor method using the Cholesterol kit. Serum sample, 200 µl was added to 200 µl of the reagent 3 (Precipitating reagent), mixed well and incubated at 37°C for 10 min. The serum mixture was centrifuged for 15 min at 2000 rpm and clear supernatant was separated. Hundred µl of the supernatant was mixed with 1000 µl reagent 1 (cholesterol reagent), incubated at 37°C for 10 min. To 1000 µl of the reagent 1 (cholesterol reagent), 100 µl of standard HDL-C (50 mg/dl) was added and incubated 37°C for 10 min The absorbance was read at 505 nm using a semi auto analyser. Concentration of HDL-C (mg/dl) was calculated using formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 50 \times 2\left(\text{dilution factor}\right)$$

Serum VLDL and LDL level were estimated as per the Freidwald formula.

VLDL = TG/5
LDL = TC– HDL – VLDL

For FC estimation, 1 ml of solution A (5 ml of formalin + 0.858 ml of concentrated HCl made up to 100 ml using distilled water) and 15 ml of solution B (1 ml of triton × 100 + 0.6 ml of Brij + 0.858 ml conc. HCl made up to 100 ml with 0.9% NaCl.) was added to 5 g of wet faeces, mixed well, centrifuged at 2500 rpm for 15 min, supernatant

was collected and cholesterol estimation was done using enzymatic kit method.

For estimation of faecal bile acids, to 1 g of wet faeces added 50 ml absolute ethanol, mixed well, covered the mouth of the conical flask with aluminium foil, kept in a boiling water bath for 20-30 min and the mixture was filtered into a round bottom flask, rinsed twice with about 5 ml hot ethanol. Ethanol was evaporated using rotary evaporator at 60-70°C at 180 rpm under vacuum. To the residue, 5 ml of 5% sodium hydroxide solution was added, boiled for 30 min, cooled to room temperature and 1 ml of conc. HCl was added drop wise along the sides of the flask. To this mixture, 10 ml of diethyl ether was added, mixed well, transferred into a separating funnel, lower layer (Congo red layer) and middle layer was discarded. The superficial layer (Bile acid layer) was collected in a beaker containing a pinch of sodium sulphate salt and kept for overnight. Sodium sulphate salt was filtered and washed once with 5 ml diethyl ether. The residue obtained after evaporation of diethyl ether was dissolved in 10 ml acetone and mixed well. Acetone was evaporated after transferring 1 ml of aliquot into a clean beaker. To the residue, 5 ml of 65% sulphuric acid was added and kept for incubation at 60°C for 15 min in a water bath. The solution was allowed to cool at room temperature and was used for estimating Cholic acid by reading the absorbance at 320 nm and Desoxycholic acid at 385 nm using Ultraviolet-Visible spectrophotometer.

Indirect Assessment of 3-Hydroxy-3-Methyl Glutaryl Co-Enzyme A Reductase Activity in Liver Tissue

Eighteen adult male rats weighing 150-200 g were divided into three groups of six animals each. Animals of the first group served as vehicle control, received 0.2% w/v CMC, 10 ml/kg p.o. Animals of the second and third group were pre-treated with Atorvastatin (7.2 mg/kg p.o) and Lipovedic (180 mg/kg, p.o.) respectively for seven consecutive days. On the 8th day the animals were sacrificed and HMG CoA reductase activity was assessed. The liver was removed as quickly as possible and a 10% w/v (10 g/dl) homogenate was prepared in saline arsenate solution. The homogenate was deproteinised using an equal volume of dilute perchloric acid and allowed to stand for 5 min, followed by centrifugation at 2000 rpm for 10 min. To 1 ml of the filtrate, 0.5 ml of freshly prepared alkaline hydroxylamine reagent (for estimation of HMG-CoA) and dilute hydroxylamine reagent (for estimation of mevalonate) was added. It was mixed and 1.5 ml of ferric chloride reagent was added after 5 min. The absorbance was read after 10 min at 540 nm versus a similarly treated saline arsenate blank. The ratio of HMG-CoA/mevalonate was calculated.[16,17]

Statistical Analysis

The results were expressed as Mean ± Standard Error of Mean and analysis was carried out by one-way ANOVA. *Post hoc* analysis was done by Tukeys multiple comparison test to estimate the significance of difference between various individual groups. $P < 0.05$ was considered statistically significant.

RESULTS

Lipovedic (180 mg/kg, p.o) decreased serum TC, TG, LDL-C, VLDL-C and increased HDL-C in triton induced hyperlipidaemic rats as shown in Table 1.

As seen from Table 2, Lipovedic decreased serum TC, TG, LDL-C, VLDL-C but increased HDL-C and FC excretion when compared to HCD control rats. There was no significant increase in the faecal excretion of cholic acid [Figure 1] and deoxycholic acid [Figure 2].

Lipovedic increased the HMG CoA/mevalonate absorbance ratio [Table 3] significantly ($P < 0.001$) as compared to the vehicle control rats. The ratio was comparable with that of the standard drug, Atorvastatin (7.2 mg/kg p.o).

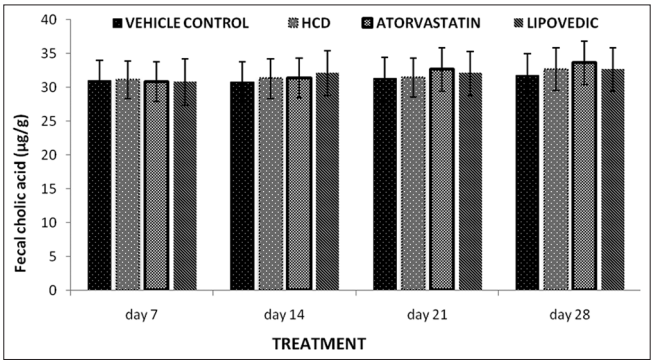

Figure 1: Effect of Lipovedic on faecal cholic acid excretion in high cholesterol diet induced hypercholesterolaemia $n = 6$, values are expressed as mean ± standard error of mean, Oneway ANOVA followed by Tukeys multiple comparison test

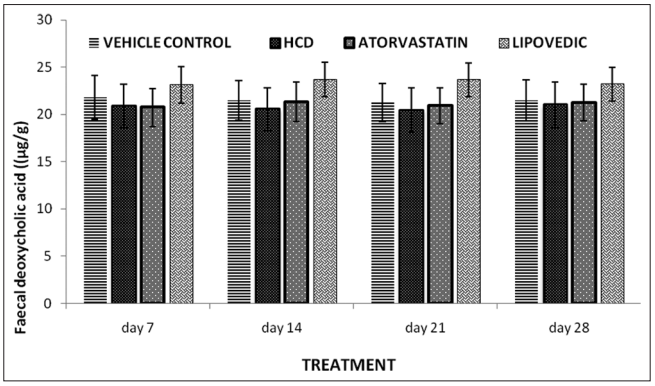

Figure 2: Effect of Lipovedic on faecal deoxycholic acid excretion in high cholesterol diet induced hypercholesterolaemia $n = 6$, values are expressed as mean ± standard error of mean, Oneway ANOVA followed by Tukeys multiple comparison test

Table 1: Effect of Lipovedic on serum TC, TG, HDL-C, LDL-C and VLDL-C in triton induced hyperlipidaemic rats

Treatment	Parameter (mg/dl)	Hour 0	Hour 18	Hour 24	Hour 40	Hour 48
Group I Vehicle control	TC	79.585±2.39	80.895±1.699	79.33±2.132	80.617±2.275	80.043±1.98
	TG	106.67±4.42	106.926±4.132	110.402±4.985	108.49±3.52	107.183±4.161
	HDL	30.661±1.06	30.918±0.985	30.263±1.017	30.435±1.065	31.105±0.963
	LDL	27.588±3.98	28.591±2.81	26.989±3.372	28.483±3.389	26.961±3.151
	VLDL	21.335±0.88	21.385±0.826	22.080±0.997	21.698±0.704	21.476±0.832
Group II Hyperlipidaemic control (triton 200 mg/kg, i.p)	TC	78.8±1.839	270.52±6.132^{+}	382.3±6.847^{+}	176.68±5.833^{+}	121.38±5.312^{+}
	TG	111.25±4.01	303.285±5.5^{+}	425.19±4.66^{+}	312.28±5.985^{+}	147.96±4.835^{+}
	HDL	31.645±0.83	25.065±1.292**	20.68±1.144^{+}	22.803±1.047^{+}	26.03±0.951**
	LDL	24.904±1.33	184.806±5.555^{+}	276.583±5.733^{+}	91.421±4.548^{+}	65.761±5.026^{+}
	VLDL	22.251±0.64	60.657±1.1^{+}	85.038±0.932^{+}	62.457±1.197^{+}	29.593±0.967^{+}
Group III Atorvastatin (7.2 mg/kg, p.o)	TC	78.882±2.78	155.09±4.719^{++}	215.34±5.659^{++}	199.08±4.65^{++}	79.068±3.01^{++}
	TG	108.94±4.01	196.827±5.816^{++}	285.121±8.093^{++}	159.76±5.63^{++}	111.92±6.07^{++}
	HDL	31.6±0.98	36.086±1.121^{++}	40.866±1.118^{++}	36±0.983+$^{+}$	32.92±0.883^{++}
	LDL	25.493±3.09	79.646±6.618^{++}	117.452±6.384^{++}	51.136±5.45^{++}	23.765±3.47^{++}
	VLDL	21.788±0.8	39.365±1.163^{++}	57.024±1.618^{++}	31.95±1.127^{++}	22.38±1.214^{++}
Group IV Lipovedic (180 mg/kg, p.o)	TC	79.545±2	177.45±5.059^{++}	253.17±7.458^{++}	141.59±7.32^{++}	100.2±4.447*
	TG	108.84±3.66	237.148±6.774^{++}	330.166±6.111^{++}	169.3±5.017^{++}	117.43±4.596*
	HDL	31.85±0.99	35.726±1.023^{++}	40.095±1.118^{++}	35.836±1.19^{++}	32.33±0.945^{++}
	LDL	25.926±2.14	94.293±6.387^{++}	147.046±8.205^{++}	71.896±7.856*	44.396±5.030$^{\#}$
	VLDL	21.768±0.73	47.429±1.354^{++}	66.033±1.222^{++}	33.86±1.003^{++}	23.487±0.919*

n=6, Values are expressed as mean±SEM; Oneway ANOVA followed by Tukeys multiple comparison test. ^{+}P<0.001; **P<0.01 versus vehicle control; ^{++}P<0.001; *P<0.01 and $^{\#}P$<0.05 versus hyperlipidaemic control; TC – Total cholesterol; TG – Triglycerides; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low density lipoprotein; HDL-C – High density lipoprotein-cholesterol; LDL-C – Low density lipoprotein-cholesterol; VLDL-C – Very low density lipoprotein-cholesterol

Table 2: Effect of Lipovedic on TC, TG, HDL-C, LDL-C, VLDL-C and FC excretion in high cholesterol diet induced hyperlipidaemic rats

Treatment	Parameter	Day 7	Day 14	Day 21	Day 28
Group I Vehicle control	TC (mg/dl)	79.11±2.832	79.596±3.144	79.828±2.977	79.598±3.255
	TG (mg/dl)	115.038±4.008	115.406±4.574	116.861±4.074	118.31±4.098
	HDL (mg/dl)	32.566±1.517	33.031±1.736	33.616±1.799	34.716±1.529
	LDL (mg/dl)	23.535±2.415	23.483±2.901	22.839±2.955	21.219±3.127
	VLDL (mg/dl)	23.007±0.801	23.0813±0.914	23.372±0.814	23.662±0.819
	FC (µg/dl)	3.55±0.166	3.59±0.159	3.57±0.161	3.54±0.154
Group II Hyperlipidaemic control	TC (mg/dl)	83.488±4.652	128.13±7.577^{a}	174.118±7.225^{a}	239.165±11.12^{a}
	TG (mg/dl)	121.485±5.507	199.42±2.72^{a}	255.7±6.892^{a}	332.851±9.294^{a}
	HDL (mg/dl)	30.596±1.376	26.546±1.466$^{\$}$	20.586±1.437^{a}	16.863±1.141^{a}
	LDL (mg/dl)	28.594±2.479	61.698±6.395^{b}	102.391±6.336^{a}	155.731±10.516^{a}
	VLDL (mg/dl)	24.297±1.101	39.885±0.544^{a}	51.14±1.378^{a}	66.570±1.858^{a}
	FC (µg/dl)	3.531±0.177	3.555±0.178	3.515±0.181	3.543±0.162
Group III Atorvastatin (7.2 mg/kg, p.o)	TC (mg/dl)	76.126±4.064	85.775±4.721^{+}	100.61±7.411^{+}	117.48±8.201^{+}
	TG (mg/dl)	112.365±5.273	138.69±5.803^{+}	158.37±6.429^{+}	176.283±7.00^{+}
	HDL (mg/dl)	34.583±1.519	40.15±1.276^{+}	44.54±1.601^{+}	49.858±2.253^{+}
	LDL (mg/dl)	19.070±4.400	17.887±5.084^{+}	24.397±8.121^{+}	32.365±9.318^{+}
	VLDL (mg/dl)	2.473±1.054	27.738±1.160^{+}	31.674±1.285^{+}	35.256±1.401^{+}
	FC (µg/dl)	3.758±0.149	4.34±0.219	4.88±0.189*	5.336±0.176^{+}
Group IV Lipovedic (180 mg/kg, p.o)	TC (mg/dl)	79.203±2.399	103.97±7.568$^{\#}$	131.325±8.377*	163.563±5.696^{+}
	TG (mg/dl)	116.093±4.472	152.801±8.132^{+}	177.626±6.726^{+}	211.99±9.246^{+}
	HDL (mg/dl)	34.168±1.322	38.908±1.459^{+}	43.753±1.84^{+}	49.303±2.146^{+}
	LDL (mg/dl)	21.816±3.021	36.171±8.164$^{\#}$	52.046±7.528^{+}	71.861±5.499^{+}
	VLDL (mg/dl)	23.218±0.894	30.560±1.626^{+}	35.523±1.345^{+}	42.398±1.849^{+}
	FC (µg/dl)	3.58±0.121	4.172±0.122	4.805±0.086*	5.33±0.0983^{+}

n=6, Values are expressed as mean±SEM; Oneway ANOVA followed by Tukeys multiple comparison test. ^{a}P<0.001; ^{b}P<0.01 and $^{\$}P$<0.05 versus vehicle control; ^{+}P<0.001; *P<0.01 and $^{\#}P$<0.05 versus hyperlipidaemic control; TC – Total cholesterol; TG – Triglycerides; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low density lipoprotein; FC – Faecal cholesterol; HDL-C – High density lipoprotein-cholesterol; LDL-C – Low density lipoprotein-cholesterol; VLDL-C – Very low density lipoprotein-cholesterol

Table 3: Effect of Lipovedic (180 mg/kg p.o.) on 3-hydroxy-3-methyl glutaryl co-enzyme a reductase activity

Group	Treatment	HMG Co A/ mevalonateabsorbance ratio
1	Vehicle control (0.2% W/V CMC, 10 ml/kg)	1.520±0.133
2	Atorvastatin (7.2 mg/kg p.o)	3.045±0.141**
3	Lipovedic (180 mg/kg p.o)	2.356±0.112*

n = 6; Values are expressed as mean±SEM; Oneway ANOVA followed by Tukeys multiple comparison test.**P<0.0001 and*P<0.001 versus vehicle control; HMG Co A–3-hydroxy-3-methyl glutaryl Co-enzyme A; W/V – Weigh/volume; CMC – Caboxy methyl cellulose

DISCUSSION

Hypercholesterolemia involves heterogeneous disorders of lipid metabolism characterized by elevated levels of plasma TC and LDL-C.[1] Currently available hypolipidemic drugs Fibrates, statins and bile acid sequestrants are the drugs for treatment hypercholesterolemia, but hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function are some of the side effects of these drugs. Herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, available locally.[18] Lipovedic is a poly herbal formulation developed by Vedic Bio-Labs Pvt Ltd, Bangalore, used in obesity and hypercholesterolemia. Earlier studies have confirmed that Lipovedic increases serum HDL and significantly decrease cholesterol, LDL, VLDL and has anti-obesity activity.[5] Hence the study was taken up to screen the antihypercholesterolemic activity and explore its mechanism. The primary mechanism is proposed by emphasizing the role of Lipovedic in cholesterol absorption, cholesterol elimination and HMG CoA reductase activity.

Triton WR-1339 is a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer, which elevates serum TC, TG, LDL-C and VLDL-C.[18] Triton causes a sharp increase in serum cholesterol within 24 h (phase I-Synthetic phase) of administration, followed by a decline nearly to control levels (phase II)[19] Increase in serum TC in phase I of triton induced hypercholesterolemia is thought to be due to increased hepatic synthesis of cholesterol. Drugs interfering with cholesterol biosynthesis are found to be active in phase I, while those interfering with cholesterol metabolism and excretion are active in phase II.[13,19,20] It is reported that triton elevates plasma TG essentially by preventing its uptake and clearance by inhibiting Lipoprotein Lipase (LPL) and Lecithin: Cholesterol acyl transferase (LCAT).[6] In our study we found that, Lipovedic (180 mg/kg, p.o) reversed the triton induced hyperlipidaemia significantly. It reduced the serum TC and TG, suggesting the drug to be interfering predominantly in phase I.[6] The results were comparable with that of the standard drug, Atorvastatin (7.2 mg/kg p.o).

The reduction in serum TC and TG may be due to some of the constituents of Lipovedic viz *C. mukul, E. officinalis, P. longum, Z. officinalis, A. heterophyllum, C. pareira.* These medicinal herbs have promising cholesterol lowering action.[7,8]

In HCD fed rats, there was an increase in serum TC, TG, LDL-C, VLDL-C and decrease in HDL-C compared to control rats. HCD increases cholesterol input in liver and hepatic synthesis of VLDL-C and LDL-C. Furthermore, it also leads to saturation, suppression and decrease in the number of hepatic LDL receptors. Elevated serum LDL-C may be due to decreased removal capacity of LDL-C by hepatic LDL receptors from the blood. Atherogenic diet elevates serum TG levels essentially by preventing its uptake and clearance by inhibiting LPL.[21] Cholic acid, a component of HCD increases cholesterol absorption by its emulsifying property and decrease cholesterol excretion by concomitant suppression of cholesterol 7 α-hydroxylase.[1] In this study, Lipovedic reduced the serum cholesterol by 31.6%, TGs by 36.31%, LDL-C by 53.86%, VLDL-C by 36.32% and increased serum HDL-C, FC excretion on day 28 as compared to the HCD control groups. This could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract.[18] Increased HDL may be due to the increase in the activity of LCAT, which play a key role in incorporating the free cholesterol into HDL and transferring back to VLDL or Intermediate Density Lipoproteins, which is taken back by the liver cells.[22] The results were comparable with that of the standard drug, Atorvastatin (7.2 mg/kg p.o).

In the present study, HMG-CoA reductase activity was indirectly measured in terms of the ratio of HMG-CoA to mevalonate. HMG-CoA was determined by its reaction with hydroxylamine hydrochloride at alkaline pH. Mevalonate was estimated by reaction with the same reagent but at pH 2.1. At this pH, the lacton form of mevalonate readily reacts with hydroxylamine hydrochloride to form the hydroxamate.[23] It was found that, Lipovedic increased the HMG CoA/mevalonate absorbance ratio significantly ($P < 0.001$) as compared to the vehicle control rats. The ratio was comparable with that of the standard drug, Atorvastatin (7.2 mg/kg p.o). The increase in the ratio indicates the suppression HMG CoA reductase which catalyses the conversion of HMG-CoA to mevalonate using NADPH (nicotinamide adenine dinucleotide phosphate) as reducing equivalent and is the major rate limiting step in cholesterol biosynthesis.[24]

CONCLUSION

Lipovedic is a potential antihypercholesterolaemic drug and is proposed to act by Figure 3. Inhibiting of HMG

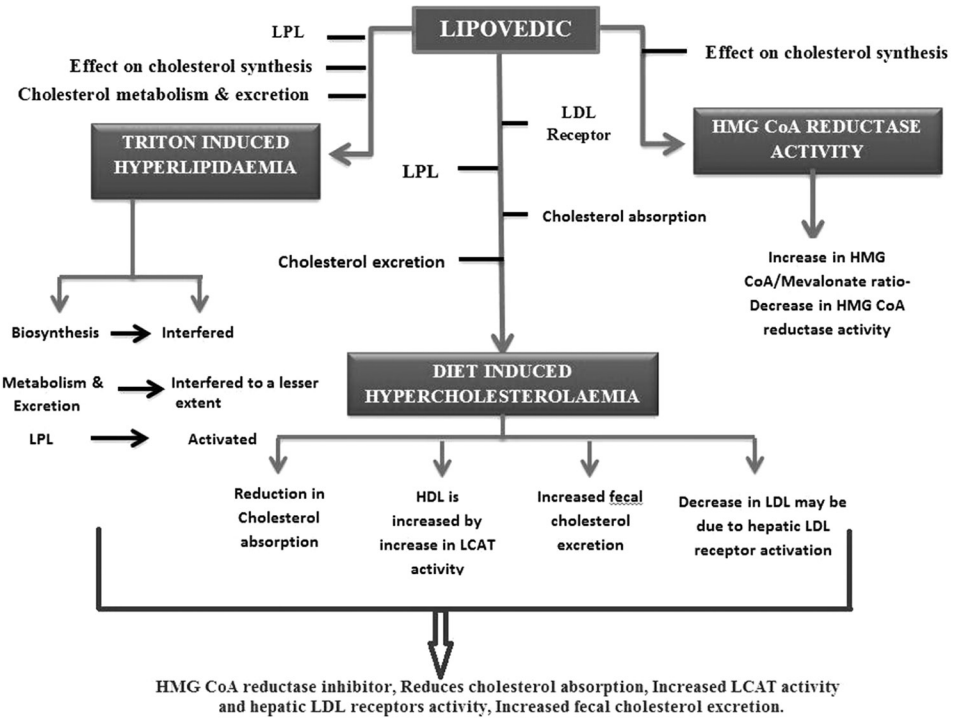

Figure 3: Schematic representation of anti-hypercholesterolaemic action of Lipovedic

CoA reductase, increasing FC excretion, reducing dietary cholesterol absorption, increased hepatic LDL receptor expression, activating LCAT potentiating LPL.

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