http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (3): 346-355, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.346.355

Evaluation of Antihyperlipidemic Potency of a Polyherbomineral Formulation (AF-LIP) in Experimental Animal Models

[1]P. Ashok, [1]D. Singh, [1]D.N. Poulose and [2]B. Suresh
[1]Department of Pharmacology, KLE University's College of Pharmacy, Bangalore, Karnataka, India
[2]Anglo French Drug and Industries Ltd., Bangalore, India

Abstract: There is a growing interest in the screening of antihyperlipidemic activity and the present study deals with the screening of a polyherbomineral formulation (AF-LIP) which possess many important ingredients reported to have antihyperlipidemic potency. Acute antihyperlipidemic activity was evaluated by using Triton WR-1339 (100 mg kg^{-1}) and chronic, induced by high fat diet. Total Cholesterol (TC), triglycerides (TG), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Very Low Density Lipoproteins (VLDL) were examined in addition to HMG-CoA reductase enzyme activity and fecal cholesterol excretion. In Triton WR-1339 (acute model) at the dose of 400 mg kg^{-1}, AF-LIP significantly lowered TC, TG, very Low Density Lipoproteins Cholesterol (VLDL-C), Low Density Lipoproteins Cholesterol (LDL-C) levels with simultaneous increase in High Density Lipoproteins Cholesterol (HDL-C) levels ($p<0.01$) at 6 and 24 h. Also there was significant reduction in TC and LDL-C levels at 48 h at the dose of 400 mg kg^{-1}. In chronic model also at the dose of 100, 200 and 400 mg kg^{-1}, AF-LIP significantly reduced ($p<0.001$) TC and LDL-C levels with increase in HDL-C levels. TG and VLDL-C levels were also not much affected. HMG-CoA reductase enzyme activity when estimated was not much decreased. Also AF-LIP showed significant reduction in atherogenic index ($p<0.01$) with significant increase in HDL/TC ratio ($p<0.01$). Fecal cholesterol excretion was significantly enhanced ($p<0.01$) in all the test doses of AF-LIP. AF-LIP may be beneficial for the treatment of atherosclerosis, since atherosclerosis is one of the secondary complications of hyperlipidemia.

Key words: Antihyperlipidaemic activity, AF-LIP, triton WR-1339, high fat diet, atherosclerosis

INTRODUCTION

It was demonstrated for the first time in 1984 that serum cholesterol level is a prevalent risk factor for Coronary Heart Disease (CHD). The risk for CHD can be brought down by 2 by a 1% drop in serum cholesterol levels (Jain *et al*., 2007). The excessive intake of triglycerides, cholesterol in diet is a hallmark for the development of the two cardiovascular risk factors, hypertriglyceridemia and hypercholesterolemia (Sheng *et al*., 2006).

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated complications. Atherosclerosis is derived from the greek words, 'athero' and 'sclerosis' which means gruel and hardness, respectively. Atherosclerosis may be defined as degenerative changes in the intima of medium and large arteries (Jain *et al*., 2007).

Coronary heart disease (CAD) is caused by the obstruction of the artery that supplies nutrients and oxygen to the heart. Generation of free radicals and elevated lipid levels in serum plays a key role in complications like CAD and atherosclerosis. A combination of risk factor like sedentary habits, stress, alcohol and tobacco have accentuated the risk for several complications associated with CAD. The incidence of CAD and other cardiovascular disorders can be reduced by dietary modification, physical exercise, abstinence from tobacco, alcohol and change in lifestyle. Active constituents like phytosterols and natural antioxidants are found to be effective in reducing elevated lipid levels and also alleviate peroxidative modification of lipoproteins and occurrence of atherosclerosis (Visavadiya and Narasimhacharya, 2005).

There are several reports on the role of natural agents in regulating serum lipid levels. Usage of plant products are considered to have less deleterious effects than synthetic agents and thus led to the discovery of new therapeutic agents from natural sources as antioxidants, hypoglycemics and hypolipidaemics. It is well established that diet rich in vegetables and fruits can reduce cardiovascular diseases (Bahramikia and Yazdanparast, 2008).

Corresponding Author: Purnima Ashok, Department of Pharmacology, KLE University's College of Pharmacy, Bangalore, Karnataka, India

AF-LIP under study is a polyherbomineral formulation containing *Commiphora mukul* as one of the ingredient which improves cardiac function, rhythm and checks on fat accumulation in the body by regulating its metabolism and also by burning excess fat. This formulation also contains *Terminalia arjuna*, *Sausshrea lappa*, *Cyperus rotundus*, *Embelica ribes*, *Berberis aristata*, *Plumbago zeylanica*, *Alllium sativm*, processed minerals and processed asphaltum.

All these together may help to reduce the cholesterol and triglycerides levels (Khanna *et al*., 1996).The present study is designed to give substantial support to the promises regarding this antihyperlipidemic polyherbomineral formulation in hyperlipidemic animal models.

MATERIALS AND METHODS

Animals: Female albino Wistar rats (150-200 g) were used for acute oral toxicity study and animals of either sex (180-220 g) were used for assessment of antihyperlipidemic activity (acute and chronic model). Animals were procured two weeks prior to study and acclimatized in our laboratory with standard diet. Animals were maintained at standard room temperature of 25±1°C, humidity 45-55 % and 12-12 h light dark^{-1} cycle with free access to commercial pelleted rat chow (Mfg: M/S Gold mohur foods and feeds, Mumbai) and water *ad libitum*.

The approval of Institutional Animal Ethics Committee (IAEC) of KLES's College of Pharmacy, Bangalore, was taken before experimentation. Animal maintenance and handling were in all accordance with internationally accepted standard guidelines for laboratory animals.

Determination of acute oral toxicity (OECD guidelines, 2001): The acute oral toxicity of AF-LIP was carried out according to Organization of Economic Cooperation and Development (OECD) guidelines 425 by using female albino Wistar rats (150-200 g) which were maintained under standard conditions. Animals were kept under fasting 12 h prior to the experiment, water given *ad libitum*. Test drug was administered to one animal in a single dose of 2000 mg kg^{-1} by gavage using a stomach tube. After observation of 1 animal, other 4 additional animals sequentially were given with same dose orally. All the animals were observed individually for signs of toxicity which includes change in skin and fur, effect on autonomic and central nervous system, somatomotor activity and behavioral changes. Others observation include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Animals observed for first four hours and thereafter for total of 14 days.

Preparation of solutions: Test drug AF-LIP and both standards drugs Simvastatin and Fenofibrate were dissolved in distilled water. All the drugs were freely soluble in water and administered orally for experimental purpose. For induction of hyperlipidemia Triton WR-1339 was administered intraperitoneally and was prepared as a solution in physiological saline. All the drugs and Triton WR-1339 were freshly prepared each time before use.

Assessment of antihyperlipidaemic activity: For antihyperlipidaemic activity of AF-LIP albino Wistar rats of either sex were used.

Antihyperlipidaemic activity was assessed by two methods:

- **Triton WR-1339 (acute model):** In this model TC, TG, HDL, VLDL and LDL were estimated in blood serum
- **Diet containing high fat (chronic model):** In chronic model along with these above mentioned parameters:
    - Estimation of HMG-CoA reductase activity
    - Estimation of fecal cholesterol excretion was also undertaken

Acute antihyperlipidemic activity (triton WR-1339 induced): Albino Wistar rats of either sex weighing 180-220 g were divided in to seven groups consisting of 6 rats in each group. The animals were divided as follows:

Group 1: Normal control, injected with saline i.p.
Group 2: Hyperlipidemic control
Group 3: Standard drug treated, Simvastatin (4 mg kg^{-1})
Group 4: Standard drug treated, Fenofibrate (20 mg kg^{-1})
Group 5: AL-LIP (100 mg kg^{-1})
Group 6: AL-LIP (200 mg kg^{-1})
Group 7: AL-LIP (400 mg kg^{-1})

All the groups except group 1 (normal control) simultaneously received a single i.p., injection of triton (100 mg kg^{-1}) to induce hyperlipidemia (Purnima and Mathuram, 2003).

Animals were divided into seven groups of 6 rats each and kept for overnight fasting before initiation of experiment. All the groups of animals received drugs and Triton as mentioned above. VLDL and LDL were calculated as follows (Friedewald *et al*., 1972):

VLDL = TG/5
LDL = Total cholesterol- TG/5-HDL

Chronic antihyperlipidaemic activity (high fat diet): Albino Wistar rats of either sex weighing 180-220 g were divided into seven groups consisting of 6 rats in each group.

The animals were divided as follows:

Group 1: Normal control, fed with normal diet
Group 2: Hyperlipidemic control
Group 3: Standard drug treated, Simvastatin (4 mg kg^{-1})
Group 4: Standard drug treated, Fenofibrate (20 mg kg^{-1})
Group 5: AL-LIP (100 mg kg^{-1})
Group 6: AL-LIP (200 mg kg^{-1})
Group 7: AL-LIP (400 mg kg^{-1})

The animals of all the groups except group 1 (fed with normal diet), were fed with high fat diet to induce hyperlipidemia for first 15 days (group 2-7).

Adult albino Wistar rats (180-220 g) of either sex were used in the study. Animals were assigned to seven groups of six animals each. From 15th day onwards until 30th day, in addition to above diet group 3-7 received standard drugs and different doses of AF-LIP as mentioned above.

Serum lipid profiles were determined on day 0 (Basal value), day 15th (Induction value) and on day 30th (Post treatment value). Blood samples were withdrawn from the animals by retro-orbital puncture under light ether anesthesia. These were centrifuged at 4500-5000 rpm to separate serum and then estimated for TC, TG, HDL-Cholesterol using commercial kits (Agappe diagnostics, India).

LDL and VLDL were calculated by using Friedewald's formula (Friedewald *et al*., 1972).

Estimation of HMG-COA reductase activity: The method estimates the HMG-CoA/mevalonate ratio as an index of activity of HMG-CoA reductase enzyme. Liver tissues were collected from all the animals as quickly as possible and 10% homogenate was prepared in saline arsenate solution. The homogenate was deproteinized using an equal volume of dilute perchloric acid and allowed to stand for 5 min followed by centrifugation. To 1 mL of the filtrate, 0.5 mL of freshly prepared hydroxylamine reagent (alkaline in case of HMG-CoA) 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 against similarly treated saline arsenate blank. The ratio of HMG-CoA/mevalonate was calculated (Rao and Ramakrishnan, 1975).

Estimation of fecal cholesterol excretion: Fecal matter was collected during the last 3 days of treatment period from all the groups. The dried and powdered fecal matter was extracted with chloroform: methanol (2: 1). The resultant extract was then analyzed for fecal cholesterol content in a manner similar to that of serum. The cholesterol excreted in the fecal matter (mg g^{-1}) was calculated (D'Souza *et al*., 2007).

Calculation of Atherogenic index (Purohit and Vyas, 2005): It is calculated as:

$$\text{Atherogenic index} = \text{LDL} + \frac{\text{VLDL}}{\text{HDL}}$$

Biochemical estimations: Blood samples were drawn from retroorbital plexus under light ether anesthesia at 0, 6, 24 and 48 h. Samples were immediately centrifuged at 4500-5000 rpm to separate serum and then estimated for TC, TG, HDL-cholesterol using commercial kits (Agappe diagnostics, India).

Accordingly, LDL-Cholesterol and VLDL-Cholesterol were calculated by using Friedewald's formula (Friedewald *et al*., 1972):

VLDL = TG/5
LDL = Total cholesterol-[TG/5]-HDL

Statistical analysis: Results are expressed as Mean±SEM and subjected to One Way Analysis of Variance (ANOVA) followed by Dunnett's test and values $p<0.05$ were considered to be significant.

RESULTS

Acute oral toxicity study of AF-LIP was done according to OECD guidelines 425. There were no signs of toxicity, behavioral and autonomic changes in all the animals when observed individually for first four hours and thereafter for total of 14 days. Animals were found to be safe upto 2000 mg kg^{-1} body weight.

***In vivo* antihyperlipidaemic activity:** The results of antihyperlipidaemic activity of AF-LIP on Triton-induced hyperlipidemia are given in Table 1.

In positive control group the rats treated with Triton WR-1339 (100 mg kg^{-1}) after 24 h of injection, TG and VLDL-cholesterol increased significantly ($p<0.01$).

Effect of simvastatin: (4 mg kg^{-1}): When compared to control group Simvastatin treated animals showed a significant reduction in TC, TG, VLDL-cholesterol and LDL-cholesterol levels ($p<0.01$) in six hour, also there was a significant rise in HDL-cholesterol level ($p<0.01$).

Table 1: Effect of AF-LIP on serum TC, TG, HDL, VLDL and LDL levels in triton WR-1339 induced hyperlipidemia

	Saline control	Triton control (100 mg kg^{-1})	Simvastatin (4 mg kg^{-1})	Fenofibrate (20 mg kg^{-1})	AF-LIP (100 mg kg^{-1})	AF-LIP (200 mg kg^{-1})	AF-LIP (400 mg kg^{-1})
TC levels							
0 h	87.94±4.87	89.61±5.84	88.55±2.82	89.54±4.22	76.54±3.45	79.23±4.46	74.16±4.39
6 h	87.97±4.55	90.40±3.70	57.22±1.74^{b**} (36.60±3.37)	58.51±2.13^{b**} (35.11±4.76)	87.50±2.80	85.02±5.14^{b*}	70.27±3.45^{b**} (22.08±6.06)
24 h	87.38±5.77	90.14±3.08	63.17±3.26^{b**} (29.85±4.22)	63.40±2.80^{b**} (29.60±3.69)	89.06±4.58	77.66±2.09^{b**}	67.95±3.37^{b**} (24.63±1.92)
48 h	88.26±4.31	89.12±3.74	81.59±2.34^{b**} (8.28±5.21)	66.46±3.12^{b**} (25.36±3.67)	87.82±3.86	83.87±4.51	65.24±5.40^{b**} (26.71±6.44)
TG levels							
0 h	101.54±2.81	98.72±2.70	128.62±6.44	112.17±9.94	112.94±6.09	113.63±4.32	105.29±3.78
6 h	98.64±5.64	138.97±5.36^{a**} [29.00±3.21]	70.72±2.04^{b**} (49.00±2.93)	76.51±3.38^{b**} (44.88±3.18)	137.82±4.74	128.42±3.76^{b*} (7.46±4.50)	111.35±10.04^{b**} (19.82±7.40)
24 h	98.82±6.86	122.05±4.69^{a**} [18.96±5.77]	91.39±2.62^{b**} (24.98±4.51)	89.85±5.21^{b**} (26.29±5.12)	132.99±6.91^{b*}	130.31±7.78	109.42±7.34^{b**}
48 h	99.54±4.27	106.29±6.80	115.08±3.94	103.02±1.56	129.38±6.58^{b**}	117.24±7.33^{b*}	102.97±12.08
HDL-C levels							
0 h	28.62±2.54	30.47±1.69	30.43±2.12	29.49±2.17	30.90±3.04	30.92±2.52	29.98±1.82
6 h	28.83±1.36	21.64±1.32^{a**} (24.72±6.60)	32.45±2.76^{b**} [33.06±4.69]	33.64±2.25^{b**} [35.27±7.33]	30.87±2.52^{b**} [29.69±4.19]	32.28±3.66^{b**} [32.32±7.57]	31.97±3.36^{b**} [31.92±5.54]
24 h	29.75±1.18	24.08±3.23^{a**} (19.15±9.32)	31.47±3.15^{b**} [22.25±16.37]	30.79±2.97^{b**} [20.94±13.98]	29.75±1.52^{b**} [18.70±12.73]	32.77±1.91^{b**} [26.49±8.90]	29.14±2.00^{b**} [16.58±15.43]
48 h	29.85±1.86	28.98±1.44	32.93±2.58^{b*} [11.54±8.35]	30.13±2.34	30.05±2.27	31.57±2.14	32.04±3.57
VLDL-C levels							
0 h	20.30±0.56	19.74±0.54	25.72±1.28	22.43±1.98	22.58±1.21	22.72±0.86	21.05±0.75
6 h	19.72±1.12	27.79±1.07^{a**} [29.00±3.21]	14.14±0.40^{b**} (49.02±2.93)	15.30±0.67^{b**} (44.98±3.18)	27.56±0.94	25.68±0.73^{b*} (7.46±4.50)	22.27±2.00^{b**} (19.82±7.40)
24 h	19.76±1.37	24.41±0.93^{a**} [18.96±5.77]	18.27±0.52^{b**} (24.98±4.51)	17.97±1.04^{b**} (26.29±13.98)	26.59±1.38^{b*}	26.06±1.55	21.88±1.46^{b**}
48 h	19.90±0.85	21.25±1.36	23.01±0.78	20.60±0.31	25.87±1.31^{b**}	23.44±1.46^{b*}	20.59±2.41
LDL-C levels							
0 h	39.00±4.64	39.39±5.13	32.40±3.18	37.62±4.37	23.04±3.32	25.58±4.33	23.11±3.04
6 h	39.41±5.03	40.95±5.02	10.62±2.50^{b**} (73.85±6.81)	9.57±2.67^{b**} (75.84±8.86)	29.07±4.55^{b**} (28.76±9.53)	27.05±5.70^{b**} (33.76±12.77)	16.02±2.93^{b**} (60.37±8.90)
24 h	37.87±6.28	41.64±5.14	13.42±3.88^{b**} (67.32±9.78)	14.64±4.43^{b**} (64.06±12.36)	32.71±3.76^{b**} (20.81±10.23)	18.82±4.61^{b**} (54.79±9.35)	16.91±3.61^{b**} (59.09±9.16)
48 h	38.49±3.00	38.87±4.74	25.64±1.65^{b**} (33.11±10.02)	15.72±4.20^{b**} (59.16±11.14)	31.89±2.77^{b**} (17.13±11.06)	28.84±4.65^{b**} (25.29±12.80)	12.61±2.70^{b**} (67.10±8.46)

Each value is Mean±SD for 6 animals, $^{a**}p<0.001$ when group II is compared with group 1. $^{b**}p<0.001$, $^{b*}p<0.05$ when compared with group 2, Values in () indicates percentage decrease. Values in [] indicates percentage increase. TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein-cholesterol, VLDL-C: Very low density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol

There was significant reduction observed in TC and LDL-cholesterol levels ($p<0.01$) when compared to control group and all the animals showed significant rise in HDL-cholesterol level ($p<0.05$) at 48 h.

Effect of fenofibrate: (20 mg kg^{-1}): When compared to control group fenofibrate treated animals showed significant reduction in TC, TG, VLDL and LDL-cholesterol levels ($p<0.01$) in six hour by, also there was significant rise in HDL-cholesterol level ($p<0.01$). Significant reduction in level of TC, TG, VLDL and LDL-cholesterol level ($p<0.01$), also there is significant rise in HDL-cholesterol levels ($p<0.01$), when compared to control group as observed by 24 h. Animals of group in 48 h showed significant reduction in TC and LDL-cholesterol levels ($p<0.001$), however results for decrease in TG, VLDL-cholesterol and increase in HDL-cholesterol were not significant.

Effect of AF-LIP at different doses: At 6 h, AF-LIP at 100 mg kg^{-1} produced a significant decrease in LDL-cholesterol level ($p<0.01$) by 28.76±9.53%. There was no significant reduction in TC, TG, VLDL-cholesterol level. There was significant rise in HDL levels ($p<0.01$) by 29.69±4.19%. At 24 h there was significant increase in TG, VLDL-cholesterol level ($p<0.05$) and a significant decrease in LDL-cholesterol level ($p<0.01$). Also HDL-cholesterol rose significantly ($p<0.01$). Significant reduction in LDL-cholesterol level ($p<0.01$) was observed at 48 h. However, a slight increase in triglyceride and VLDL-cholesterol level was observed when compared to control group (Triton treated).

AF-LIP at the dose of 200 mg kg^{-1}, at 6 h showed a slight decline in TC levels. TG, VLDL and LDL-cholesterol levels were reduced significantly $p<0.05$, $p<0.05$, $p<0.01$. HDL-Cholesterol rose significantly ($p<0.01$) by 32.32±7.57%. After 24 h, TC and LDL-cholesterol

(by 54.79±9.35%) levels reduced significantly ($p<0.01$). HDL-cholesterol significantly increased ($p<0.01$) by 26.49±8.90%. However, there was a slight increase in TG and VLDL-cholesterol levels but not significant compared to group 2 (Triton treated). TG and VLDL-cholesterol levels increased significantly ($p<0.05$) in 48 h when compared to control group but LDL-cholesterol level reduced significantly ($p<0.01$).

AF-LIP at the dose of 400 mg kg^{-1} decreased TC, TG, VLDL and LDL-cholesterol levels significantly ($p<0.01$) and it was found to be 22.08±6.06 and 60.37±8.90% for TC and LDL, respectively. There was a significant increase in HDL-cholesterol ($p<0.01$) by 31.92±5.54% observed in 6 h. At 24 h, TC and LDL-cholesterol levels reduced significantly ($p<0.01$) by 24.63±1.92 and 59.09±9.16%, respectively. There was significant increase in HDL-C levels ($p<0.01$). After 48 h, only TC and LDL-cholesterol level were reduced significantly ($p<0.01$) by 26.71±6.44 and 67.10±8.46%, respectively. There was no significant decrease in TG, VLDL and HDL-cholesterol levels.

AF-LIP at the dose of 400 mg kg^{-1} seems to be most effective in reducing the elevated lipid levels in Triton WR-1339 induced hyperlipidemia.

From the data obtained it was also evident that AF-LIP was significantly effective at a dose of 400 mg kg^{-1} in elevating HDL-cholesterol and reducing TC, TG, VLDL and LDL-cholesterol levels comparable to standard drugs Simvastatin and Fenofibrate.

High fat diet induced hyperlipidemia: There was a significant increase in TC levels ($P<0.001$) in all groups of animals i.e., group 2-5, group 6 and 7 fed with high fat diet for 15 days. TG levels were significantly increased in group 2 (High fat diet treated), group 3 (Simvastatin treated-std 1) and group 4 (AF-LIP 200 mg kg^{-1}) $p<0.001$, $p<0.01$, $p<0.001$. But there was no significant increase in the TG levels of group 4 (Fenofibrate treated-std 2), group 5 (AF-LIP 100 mg kg^{-1}) and group 7 (AF-LIP 400 mg kg^{-1}). Similarly there was no much change in HDL-cholesterol levels except for group 5 (AF-LIP 100 mg kg^{-1}) and group 7 (AF-LIP 400 mg kg^{-1}) where there was a decrease. In these groups HDL-C levels reduced ($p<0.001$). VLDL-cholesterol levels were not significantly increased in groups except in group 2 (High fat diet treated), group 3 (Simvastatin treated-std 1) ($p<0.001$ and $p<0.01$).

Also there was a significant increase ($p<0.001$) in the levels of LDL-cholesterol in all the groups i.e., group 2-7, except group 1 (Normal control). The results of antihyperlipidaemic activity of AF-LIP on high fat diet induced hyperlipidemia are as shown in Fig. 1a-e.

Group 2 (only high fat diet) showed significant increase in TC ($p<0.001$), TG ($p<0.05$), LDL-cholesterol ($p<0.001$) and a significant decrease in HDL-cholesterol levels ($p<0.05$). Simvastatin (4 mg kg^{-1}) treated animals in group 3 showed significant reduction in TC levels ($p<0.001$). It also reduced TG ($p<0.01$), VLDL-cholesterol ($p<0.01$) and LDL-cholesterol ($p<0.001$). But there was no significant increase in HDL-cholesterol levels. Fenofibrate (20 mg kg^{-1}) significantly reduced TC levels ($p<0.001$). It also reduced TG ($p<0.01$) and VLDL-cholesterol ($p<0.01$) and LDL-cholesterol levels ($p<0.001$). But there was no significant increase in HDL-cholesterol levels.

All the three test doses 100, 200 and 400 mg kg^{-1} of AF-LIP significantly reduced TC levels ($p<0.001$) especially 200 and 400 mg kg^{-1} reduced TC levels by 27.25±9.28 and 28.51±8.07%, respectively. But there was no significant reduction in TG levels. All the three test doses of AF-LIP significantly increased HDL-cholesterol levels $p<0.001$, $p<0.05$ $p<0.001$ particularly at 200 and 400 mg kg^{-1} by 25.57±13.43 and 29.25±10.83%, respectively. There was no significant increase in VLDL-cholesterol level observed in all the animals treated with AF-LIP during induction and also in post treatment period. All three doses of AF-LIP significantly reduced elevated LDL-cholesterol level ($p<0.001$) by 27.68±7.75, 45.35±13.94 and 53.01±10.36%, respectively.

.

Effect on HMG-CoA activity: The ratio of absorbance of HMG-CoA and mevalonate is taken as an index of activity of HMG-CoA reductase enzyme. This ratio indicates biosynthesis of cholesterol in clinical condition; higher the ratio, lower is the activity of enzyme and vice versa. But there was no significant increase of this ratio in all groups except group III (Simvastatin treated-std 1) that showed a significant increase ($p<0.05$).

Effect on fecal cholesterol excretion: Both standard drugs simvastatin (4 mg kg^{-1}) and fenofibrate (20 mg kg^{-1}) induced significant ($p<0.01$) increase in the excretion of fecal cholesterol. The three test doses of AF-LIP 100, 200 and 400 mg kg^{-1} significantly increased ($p<0.01$) the fecal cholesterol excretion by 19.84±0.63, 20.53±0.60 and 22.67±1.17, respectively compared to HFD control (10.61±1.56). Test dose 400 mg kg^{-1} of AF-LIP induced maximum excretion of cholesterol ($p<0.01$) comparable to Simvastatin treated group as shown in Fig. 2.

Effect on HDL/TC ratio: HDL/TC ratio was also calculated in all the groups which shows the risk of atherosclerosis. Group 3 (Simvastatin treated-std 1)

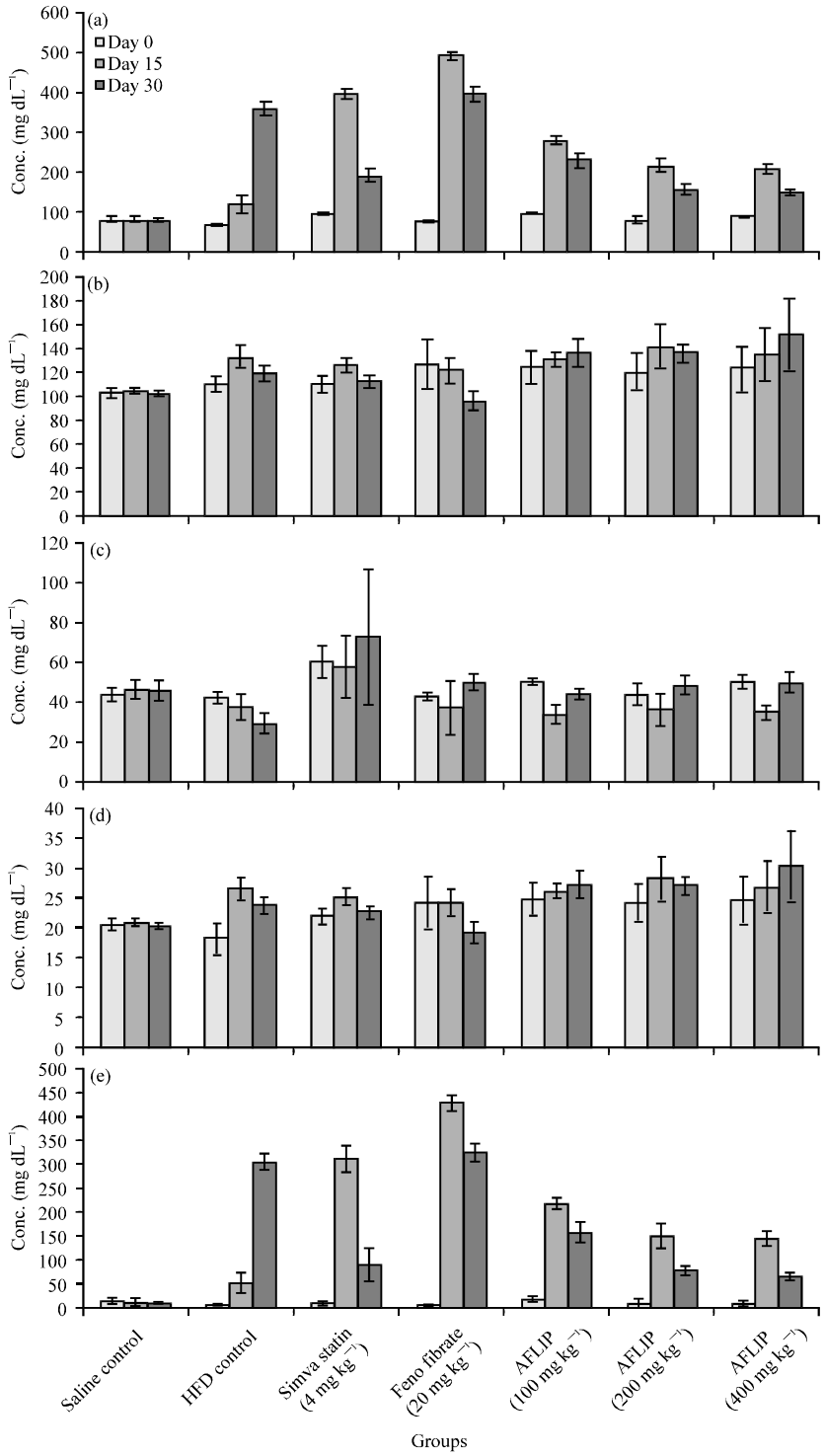

Fig. 1(a-e): Effect of AF-LIP on serum TC, TG, HDL, VLDL and LDL levels in high fat diet induced hyperlipidemia (a) Effect of AF-LIP on serum TC, (b) Effect of AF-LIP on serum TG, (c) Effect of AF-LIP on serum HDL-C, (d) Effect of AF-LIP on serum VLDL-C and (e) Effect of AF-LIP on serum LDL-C

showed a significant increase in ($p<0.01$) in HDL/TC ratio and a significant decrease in ($p<0.01$) in atherogenic index. Test dose 100, 200 and 400 mg kg^{-1} of AF-LIP significantly increased the HDL/TC ratio $p<0.005$, $p<0.01$

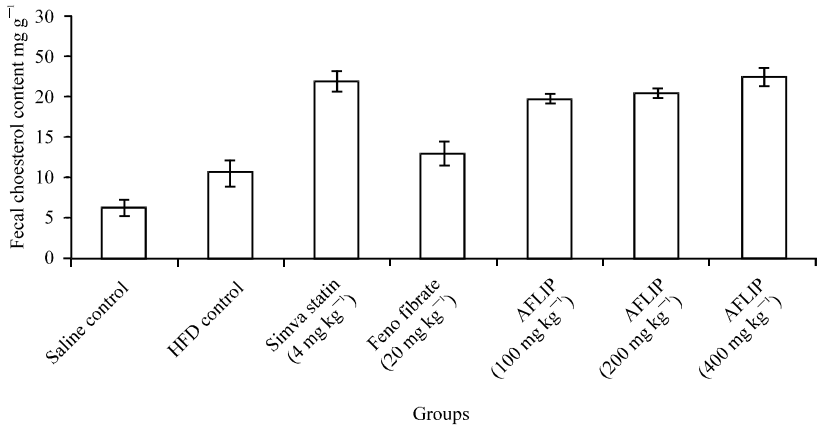

Fig. 2: Effect of AF-LIP on fecal cholesterol excretion

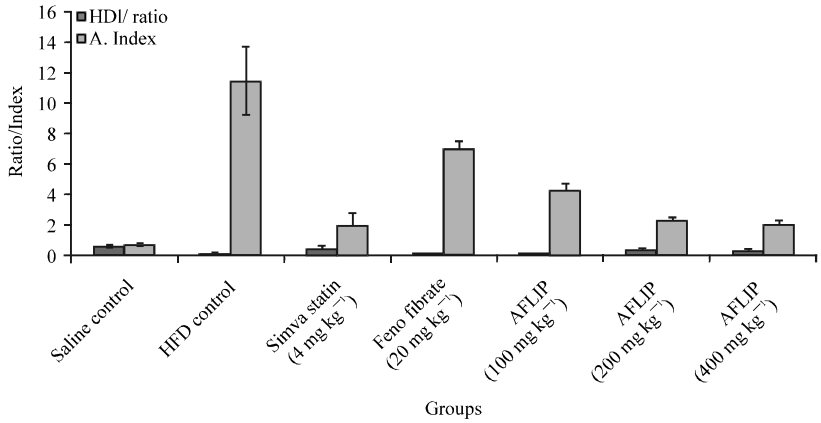

Fig. 3: Effect of AF-LIP on HDL/TC ratio and atherogenic index

and $p<0.01$, respectively which was confirmed by simultaneous decrease in the atherogenic index ($p<0.01$) by 4.20±0.60, 2.20±0.32 and 1.98±0.27, respectively compared to HFD control group (11.45±2.24). Group 4 (Fenofibrate treated) animals has not produced any significant change in HDL/TC ratio. But there was a significant decrease in atherogenic index when compared to high fat diet treated control group ($p<0.01$) as shown in Fig. 3.

DISCUSSION

Triton WR-1339 has been widely used to block clearance of triglyceride rich lipoproteins to induce acute hyperlipidemia in several animals. This model is widely used for a number of different aims and in particular in rats it has been used for screening natural or chemical hypolipidemic drugs, due to convenience in terms of length of treatment period and handling.

There are reports that many plants such as *Vaccinum myrtillus* and *Phyllanthus niruri* have been investigated for their acute hypolipidaemic activity in Triton WR-1339 induced hyperlipidemic animals (Harnafi *et al*., 2007).

Purnima and Mathurani (2003) have demonstrated that *Trichilia connaroids* (W. and A.) Bentilezen produced antihyperlipidaemic activity in Triton- induced hyperlipidemic rats.

The mechanism of the Triton-induced hypercholesterolemia includes two phases of which phase 1 is thought to be due to increased hepatic synthesis of cholesterol. Drugs interfering with cholesterol biosynthesis were shown to be active in phase 1 while drugs interfering with cholesterol excretion and metabolism were active in phase 2 (Purnima and Mathurani, 2003).

Since, AF-LIP is a polyherbomineral formulation having a bunch of herbs with many important active constituents like flavonoids, phenols, phytosterols, terpenoids, hydrolysable tannins, etc, the present claim of this formulation under study as an antihyperlipidaemic drug may be attributed to these active principles solely or synergistically.

AF-LIP in acute model (Triton) at the dose of 400 mg kg^{-1} produced a significant fall in TC, TG, VLDL-C, LDL-C levels ($p<0.01$) within 48 h of induction of hyperlipidemia. So it is thought to interfere both with cholesterol biosynthesis (phase 1) as well as with excretion and metabolism (phase 2).

In the present study Simvastatin (4 mg kg^{-1}) was used as a standard for its potent hypolipidaemic activity with known mechanism of action including inhibition of HMG-CoA reductase enzyme responsible for cholesterol biosynthesis and resultant increase in LDL receptors (Verd *et al*., 1999). In addition, Atorvastatin, belonging to a class of statins possesses an inhibitory effect on LDL oxidation (Hirunpanich *et al*., 2006).

The maximum reduction of plasma total cholesterol by AF-LIP at the dose of 400 mg kg^{-1} was similar to that of simvastatin (standard) and was associated with a decrease of its LDL fraction which is the target of several hypolipidaemic drugs. This result suggests that cholesterol lowering activity of AF-LIP could be due to rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids or due to decrease in the activity of HMG-CoA reductase enzyme responsible for cholesterol biosynthesis.

Since, the present drug under study was also effective in phase 1 (biosynthesis phase) it was investigated further for the estimation of influence on enzyme involved in biosynthesis of cholesterol.

In triton-induced hyperlipidemia the increase in plasma triglycerides might be due to an increase of VLDL secretion by the liver accompanied by the strong reduction of VLDL and LDL catabolism (Harnafi *et al*., 2007). Fibrates cause a marked reduction in circulating VLDL-C and hence triglycerides, with a modest (approximately 10%) reduction in LDL-C and 10% increase in HDL-C.

AF-LIP at the dose of 400 mg kg^{-1} markedly reduced the levels of TG similar to standard drug fenofibrate. These results suggest that AF-LIP is able to restore, at least partially catabolism of β-lipoproteins (LDL-C) and pre β-lipoproteins (VLDL-C). The restoration of catabolic metabolism of VLDL could be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase.

Present study clearly demonstrates that AF-LIP at 100, 200 and 400 mg kg^{-1} dose levels produced elevated HDL-C levels within 24 h of induction of hyperlipidemia. Hyperlipoproteinaemia with increased concentrations of cholesterol and triglycerides carrying lipoproteins is considered to be the cause of atherosclerosis with its duel sequel of thrombosis and infarction. HDL-C promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to liver, therefore increased levels of HDL-C is desirable. On the contrary, high levels of VLDL-C and LDL-C promote atherosclerosis. Therefore antiatherosclerotic drug should reduce VLDL-C and LDL-C and elevate HDL-C levels (Vogel and Vogel, 2002).

The possible mechanism of action of AF-LIP may be due to enhanced activity of LCAT (Lecithin-cholesterol acyl transferase) and inhibition of the action of hepatic TG-lipase on HDL which may contribute for a rapid catabolism of blood lipids through extra hepatic tissues. The increased HDL-C facilitates the transport of triglyceride or cholesterol from serum to liver where it is catabolised and excreted out of the body.

Since, AF-LIP reduced VLDL-C and LDL-C levels and enhanced HDL-C levels it may be beneficial in the treatment of atherosclerosis, one of the secondary complications of hyperlipidemia.

Medicinal plants have been considered as promising resources for the discovery of new drugs for hyperlipidemia. Hence AF-LIP, a polyherbal formulation was evaluated in high fat diet induced hyperlipidemia.

In this chronic model serum TC, TG, VLDL-C and LDL-C levels were increased which are the causative agents to promote atherosclerosis and other cardiovascular diseases. LDL carries cholesterol from liver to the peripheral cells and smooth muscle cells of the arteries. A rise in LDL-C may cause deposition of cholesterol in arteries and aorta and hence it is direct risk factor for CHD. The search for hypolipidaemic drugs follows the rationale that high levels of serum cholesterol are associated with an increase incidence of CHD. Reduction in LDL-C and increase in HDL-C concentrations are significantly related to lipid lowering therapy. Cholesterol, in spite of being an essential structural element of the biological membranes and precursor of many compounds such as synthesis of bile acids, steroid hormones and vitamin D, if elevated in serum could increase the risk of CHD (Bahramikia and Yazdanparast, 2008).

The present study demonstrated that rats fed with high-fat diet showed a higher concentration of serum TC, TG, VLDL-C and LDL-C compared to rats fed a standard laboratory diet. While oral administration of AF-LIP at different doses to hyperlipidemic animals at 100, 200 and 400 mg kg^{-1} significantly reduced the high level of TC.

It is widely accepted that elevation of plasma LDL-C levels are major risk factors for CHD. Direct correlation between LDL-C and atherosclerosis and also the reversibility of the related pathological events by lowering the serum level of LDL have already been reported by many research groups (Bahramikia and Yazdanparast,

2008). Our data indicated that, the high concentration of LDL-C in hyperlipidemic rats were significantly reduced by oral administration of AF-LIP.

Therefore, AF-LIP might contribute for the treatment of atherosclerosis by lowering serum LDL-C levels.

Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. HDL-mediated reverse cholesterol transport from arterial wall foam cells to liver is thought to be primary mechanism by which HDL protects from atherosclerosis. Therefore, it relates the reduced risk of atherosclerosis to an increase in HDL-C level (Bahramikia and Yazdanparast, 2008).

Our present results clearly show that AF-LIP is capable of increasing the serum levels of HDL-C in hyperlipidemic animals.

The biological oxidation processes are assisted by free radicals and their substrates, Polyunsaturated Fatty Acids (PUFA). Thus, a reduction of PUFA is likely to have a significant effect on the oxidation of LDL. Variations in the lipid composition of LDL, by reducing triglycerides, cholesterol contents could decrease LDL oxidation thereby preventing the deleterious effects followed (Hirunpanich *et al*., 2006). Further studies are required to establish the effect of AF-LIP on the lipid composition or the protective effect on the endogenous antioxidant in LDL particles.

HMG-CoA reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway. It converts HMG-CoA to mevalonate (Rao and Ramakrishnan, 1975). In the present study, HMG-CoA reductase activity was indirectly measured in terms of the ratio between HMG CoA and mevalonate. The ratio is inversely proportional to HMG-CoA reductase activity, meaning that an increase in the ratio will indicate a decrease in the enzyme activity. The rats of group fed with high fat diet showed increase in HMG CoA/mevalonate ratio as compared to rats of group 1 after 30 days of treatment. This is mainly due to inhibition of reductase activity by the exogenous cholesterol in the diet.

The AF-LIP treated rats did not show significant reduction in the activity of HMG-CoA reductase, indicating that there is no influence of AF-LIP on this enzyme. On the other hand Simvastatin (HMG-CoA reductase inhibitor) showed a significant decrease in the activity of this enzyme.

There was significant increase in the cholesterol content of the fecal matter of animals treated with AF-LIP at different doses in chronic model of hyperlipidemia indicating that the AF-LIP may promote the excretion of cholesterol.

So, it can be concluded that cholesterol reducing effect of AF-LIP in chronic hyperlipidemic model might not be due to the inhibition/reduction in the activity of HMG-CoA enzyme but it may be due to increased fecal cholesterol excretion and by some other mechanism like increased uptake by the tissues.

A significant fall in HDL-C to total cholesterol ratio was observed in high fat diet treated rats (group 2). Low levels of HDL-C are associated with high risk of coronary heart diseases. HDL participates in reverse cholesterol transport transferring cholesterol from tissue back to the liver. This action aids efflux of cholesterol from the arterial wall. HDL-C may also inhibit atherosclerosis by carrying enzyme that are antioxidants which may block early steps in atherogenesis and slow progression of lesions (Purohit and Vyas, 2005). AF-LIP at all the doses 100, 200 and 400 mg kg^{-1} tested retained this ratio comparable to normal animals, almost similar to the standard drug simvastatin by increasing HDL-C concentration.

Atherogenic index is believed to be an important factor for diagnosis of atherosclerosis. Our data clearly indicated that AF-LIP in all the three doses tested showed decrease risk factor for atherosclerosis after 15 days of drug treatment and found to be maximum at 400 mg kg^{-1}.

It has been reported that low levels of triglycerides are associated with lower risk of CHD (Bahramikia and Yazdanparast, 2008). But in our study administration of all three test doses of AF-LIP did not significantly lower the serum TG levels.

AF-LIP might have acted on the liver thereby increasing metabolism and hastening the process of excretion of excess lipids, contributing to its antihyperlipidaemic effect.

From these results it can be concluded that AF-LIP could have a potentially beneficial effect in the atherosclerosis associated with hyperlipidemia. Further work will be focused at cardioprotective and anti-atherosclerotic properties of AF-LIP.

CONCLUSION

In conclusion, the significant antihyperlipidemic activity of the polyherbal formulation AF-LIP under study in both acute and chronic models may be due to its synergistic action of its individual medicinal herbs and minerals. AF-LIP may be modifying the lipid profiles to the better by increasing the fecal cholesterol excretion rather than its influence on HMG CoA reductase enzyme. Therefore, it can be concluded that AF-LIP may be beneficial in secondary complications of hyperlipidemia like CAD and atherosclerosis.

ACKNOWLEDGMENT

The authors are thankful to M/S Anglo French Drug and Industries Ltd., Bangalore.

REFERENCES

Bahramikia, S. and R. Yazdanparast, 2008. Effect of hydroalcoholic extracts of *Nasturtium officinale* leaves on lipid profile in high-fat diet rats. J. Ethanopharmcol., 115: 116-121.

D'Souza, T., S.A. Mengi, S. Hassarajani and S. Chattopadhayay, 2007. Efficacy study of the bioactive fraction (F-3) of *Acorus calamus* in hyperlipidemia. Indian. J. Pharmacol., 39: 196-200.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of low-density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Harnafi, H., N. El-Houda-Bouanani, M. Aziz, H.S. Caid, N. Ghalim and S. Amrani, 2007. The hypolipidaemic activity of aqueous *Erica multiflora* flowers extract in Triton WR-1339 induced hyperlipidemic rats: A comparison with fenofibrate. J. Ethnopharmacol., 109: 156-160.

Hirunpanich, V., A. Utaipat, N.P. Morales, N. Bunyapraphatsara, H. Sato, A. Herunsale and C. Suthisisang, 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. J. Ethnopharmacol., 103: 252-260.

Jain, K.S., M.K. Kathiravan, R.S. Somani and C.J. Shishoo, 2007. The biology and chemistry of hyperlipidemia. Bio. Med. Chem., 15: 4674-4699.

Khanna, A.K., N.K. Kapoor and R. Chander, 1996. Lipid lowering activity of guggulsteron from *commiphora mukul* in hyperlipidemic rats. Phytother. Res., 10: 508-511.

OECD Guidelines, 2001. Guidance document on acute oral toxicity testing. Series on Testing and Assessment No. 24, Organisation for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications, Paris. http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2001)4&doclanguage=en

Purnima, A.L. and V. Mathurani, 2003. Hypocholesterolemic and antihypercholesterolemic activity of extracts of *Trichilia connaroides* on rats. Indian J. Pharma. Sci., 65: 537-539.

Purohit, A. and K.B. Vyas, 2005. Hypolipidaemic efficacy of *Capparis decidua* fruit and shoot extract in cholesterol-fed rabbits. Indian J. Exp. Biol., 43: 836-866.

Rao, A.V. and S. Ramakrishnan, 1975. Indirect assessment of hydroxymethylglutaryl-coa reductase(NADPH) activity in liver tissue. Clin. Chem., 21: 1523-1525.

Sheng, L., Z. Qian, S. Zheng and L. Xi, 2006. Mechanism of hypolipidemic effect of crocin in rats: Crocin inhibits pancreatic lipase. Eur. J. Pharmacol., 543: 116-122.

Verd, J.C., C. Peris, M. Alegret, C. Diaz and G. Hernandez *et al*., 1999. Different effect of simvastatin and atorvastatin on key enzymes involved in VLDL synthesis and catabolism in high fat/cholesterol fed rabbits. Br. J. Pharmacol., 127: 1479-1485.

Visavadiya, N.P. and A.V.R.L. Narasimhacharya, 2005. Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesteremic rats. Indian J. Pharmacol., 37: 376-380.

Vogel, H.G. and W.H. Vogel, 2002. Drug Discovery and Evaluation: Pharmacological Assay. 2nd Edn., Springer Verlag, Berlin, Germany, Pages: 1103.