ANTI-HYPERLIPIDEMIC ACTIVITY OF A POLY HERBAL FORMULATION IN EXPERIMENTAL MODELS

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Summary

A number of natural products are used in the traditional medical systems for the treatment of various diseases is gaining importance. The aim of this study is to conduct reverse pharmacology studies on a clinically used poly herbal formulation for the management of dyslipidemia. Altered lipid profile is known to elicit obesity associated with cardiovascular complications. A poly herbal formulation containing *Nelumbo nucifera, Terminalia chebula, Zingiber officinale, Glycyrrhiza glabra, Hibiscus rosasinensis, Eclipta Alba, Rosa damascena, Quercus infectoria and Hemidesmus indicus* have folkloric claim in the management of dyslipidemia. In the present study, attempts were made to provide experimental evidences by conducting reverse pharmacology studies supporting its clinical use. Dyslipidemia was induced by high cholesterol diet. Body weight (BW), total cholesterol (TC), triglycerides (TGL), low density lipoproteins (LDL), High Density lipoproteins (HDL), very low density lipoproteins (VLDL), TC/HDL ratio, LDL/HDL ratio were measured using standard procedures. Changes in the histopathological morphology in aorta, liver and heart were examined. Atorvastatin 5mg/kg/ body weight was used as standard. 0.5, 1 and 2 g/kg of the poly herbal formulation were used for investigation. The results showed that high cholesterol diet increased TC (17%), TGL (27%), LDL (56%),VLDL (25%) and modified the LDL/HDL, TC/HDL ratios to 0.9 and 2.43 respectively. A 24% gain in body weight was observed besides alteration in the histopathology aorta and liver but not in the heart. The poly herbal formulation significantly reduced the high cholesterol diet induced changes *viz.* TC by 28%, TGL by 19%, LDL by 41%, VLDL significantly. Significant increase in HDL was evident. The histopathological changes induced by high cholesterol fed diet were almost reversed to near normal in poly herbal formulation treated animals. From the above findings, we conclude the reverse pharmacology results support the folk loric claim of the poly herbal formulation for its use in dyslipedemia.

Key words: Polyherbal formulation, dyslipedemia, reverses pharmacology and cholesterol

Introduction

The use of traditional medicine on holistic approach date backs between 4000 BC to 1600 BC written in Rig Veda. Until late 1980's the scientific approach to the use of holistic medicine is based on identifying the active principle responsible for their folkloric claim. However, World Health Organization suggested that scientific validation of the traditional medicine in use is warranted to support their clinical use. Since then the use of alternative system medicines are explored for the most common diseases like obesity, hypertension, diabetes mellitus, anti-HIV, which require life long therapy in modern medicine. The possibility of use of herbal food supplements in these situations is a welcome change globally. In the present study, a formulation (Rumi Herbals, Chennai), containing *Nelumbo nucifera, Terminalia chebula, Zingiber officinale, Glycyrrhiza glabra, Hibiscus rosasinensis, Eclipta Alba, Rosa damascena, Quercus infectoria and Hemidesmus indicus* that have a claim for the beneficial effects in obesity was investigated to provide scientific validation. The ingredients have ancient claim for their efficacy in the management of hyperlipidemia (1,2).

One among the many contributing factors to the obesity is genetic component (3). The World Health Organization identified malfunction of certain biochemical parameters attributing to obesity. They are disorders of very low-density lipoproteins and chylomicrons, hyper triglycerdaemia disorders of low-density lipoprotein, high-density lipoprotein and combined hyperlipidemia. In certain circumstances hyperlipidemia may be secondary due to hypothyroidism, alcohol dependence and / or renal insufficiency. Among the lipid profile low density lipoprotein (LDL) derived especially from very low density lipoprotein (VLDL) contribute significantly to obesity and its associated disorders (4).

The management of hyperlipidemia comprises of drug therapy with or without life style modification. In fact, currently the American Heart Association has developed a step I diet (5) indicating diet control is a major factor in the management of hyperlipidemia. Common drugs those are currently available are statins and fabric acid derivatives besides omega-3-marine triglycerides for therapy of hyperlipidemia (6). Pharmacotherapy requires regular medical monitoring. The suggestions to use herbal food supplements *sans* side effects deserve considerations. One such poly herbal formulation with proved clinical efficacy (7) was identified for scientific validations through reverse pharmacology in the present study.

Materials and Methods

Drugs and Chemicals

The identified poly herbal formulations contained the following ingredients viz. p*etals of* Nelumbo *nucifera, petals of* Hibiscus *rosa sinensis, roots of* Hemidesmus *indicus, petals of* Rosa *alba, Rhizome of* Zingiber *officinale, whole plant of* Eclipta *alba, fruits of* Terminalia *chebula, roots of* Glycerrhiza *glabra, gall of* Quercus *infectoria. Rumi herbal Chennai* prepared the formulation as powder with lactose for reverse pharmacology studies. Atorvastatin (Mankind Pharma.

Ltd.), cholesterol 1% Cholic acid 0.5% (Sigma chemicals, Scientific &Co., Pondicherry), Coconut oil, (parachutes) biochemical test kits (Agape diagnostics Pvt Ltd, kerala) Animal standard pellet (Armrut rat feed chakan oil mills Ltd., Maharashtra).

Animals

Thirty adult Wistar male rats weighing between 150-250 grams were used in this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw. Institutional animal ethical committee approved experimental protocol.

Preparation of the extract

The powder of poly herbal formulation was soaked in de-mineralized and UV treated water for 8 hrs followed by boiling 2 hrs. The filtrate was concentrated on vacuum into a paste. This was stored in desiccators and used for experimental study. Twenty ml of the extract was considered equivalent to 5grams of the powder.

Induction of hyperlipidemia

Animals were fed with high cholesterol diet for 120 days (1). Animals were divided in to five groups of 6 each. All the animals in the five groups received high cholesterol diet. Besides, group I which received saline 10ml/kg, group II received Atorvastatin 5mg / kg; group III, IV and V received 0.5, 1 and 2 g/kg of poly herbal formulation respectively. At the end of 120 days, the animals were weighed, blood was collected by sinus puncture under ketamine anesthesia and serum was separated for various biochemical estimations. Immediately, animals were sacrificed by excess ketamine administration, organs such as aorta, heart and liver were examined and preserved in 10% formalin for histopathological studies. The following lipid profiles were studied (8,9).

Serum total cholesterol (TC)- (cholesterol oxidase peroxidase-cop method), Serum triglycerides (TGL)-(glycerol phosphate oxidase-GPO-method), High-density lipoprotein (HDL)-phosphotungstic method, Low-density lipoprotein (LDL)-(TC-HD-TGL/5, Freedwald formula), Very low density lipoprotein (VLDL)-TGL/5 were studied using auto analyzer. LDL/HDL Ratio and TC/ HDL Ratio were calculated (10). The lipid profiles were analyzed on day zero and on day 120.

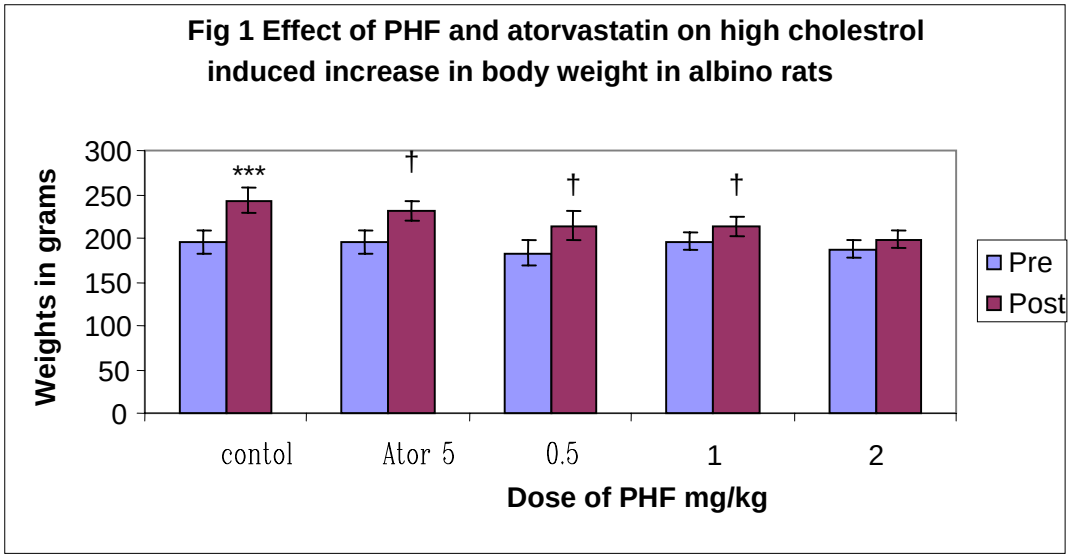
Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's t-test. P values <0.05 were considered significant.

Results

Body Weight:

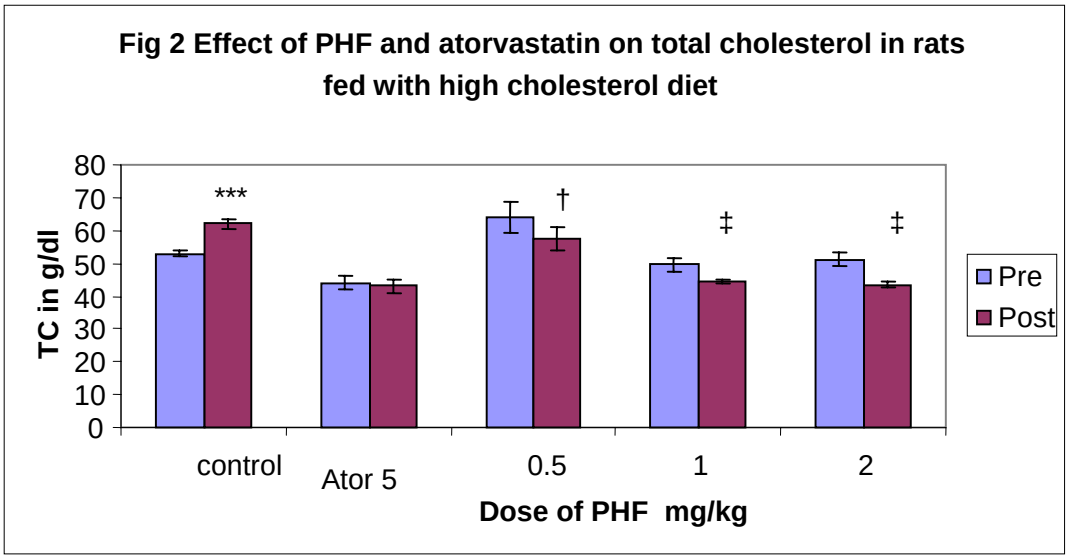
High cholesterol diet elicited 24% increase in the body weight. Atorvastatin significantly reduced the increase in body weight (to 6 %) produced by high cholesterol diet. Similarly, in poly herbal formulation treated animals a dose related decrease in the body weight when compared with that observed in high cholesterol induced diet (ranging from 7% to 19%) was observed (Figure 1). The increase in body weight produced by high cholesterol diet in control animals was considered as 100% for comparing other data



Each value represents the mean ± SEM of six experiments
†p <0.05, ‡p value<0.02 when compared with control value and ***p<0.01 when compared with the corresponding pre-treatment value.

Lipid profile:

In animals those received only high cholesterol diet, the total cholesterol increased by 17%. Atorvastatin antagonized this increase. High cholesterol diet was unable to increase and in these animals a reduction in total cholesterol (28%) compared to day zero total cholesterol in animals was observed. In animals which received poly herbal formulation a dose related decrease was recorded (Figure. 2).



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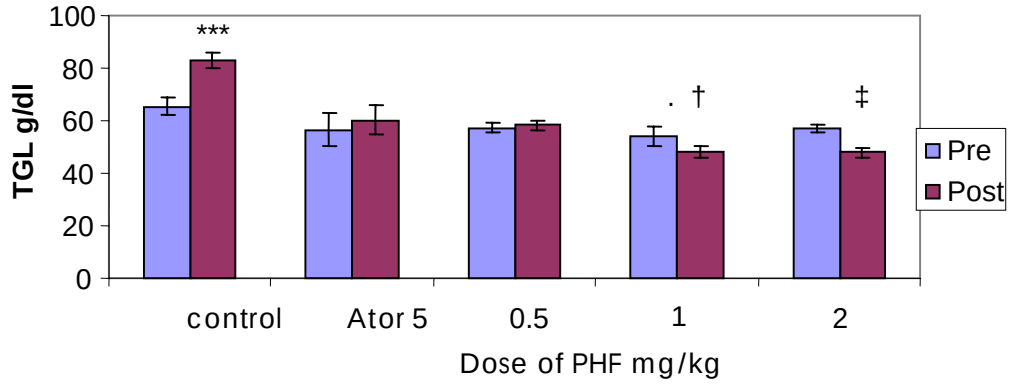
Serum Triglycerides (TGL) and Low-Density Lipoprotein (LDL):

Similar to total cholesterol, TGL and LDL levels were increased in high cholesterol diet fed animals (TGL 27% and LDL 56%) which were antagonized by atorvastatin significantly. In contrast, the poly herbal formulation reduced these parameters below the value observed in high cholesterol fed animals. (TGL 19%, LDL 41%) in a dose related manner (Figures, 3 & 4).

Fig 3 Effect of PHF and atorvastatin on triglycerides in rats fed with high cholesterol diet



Each value represents the mean ± SEM of six experiments
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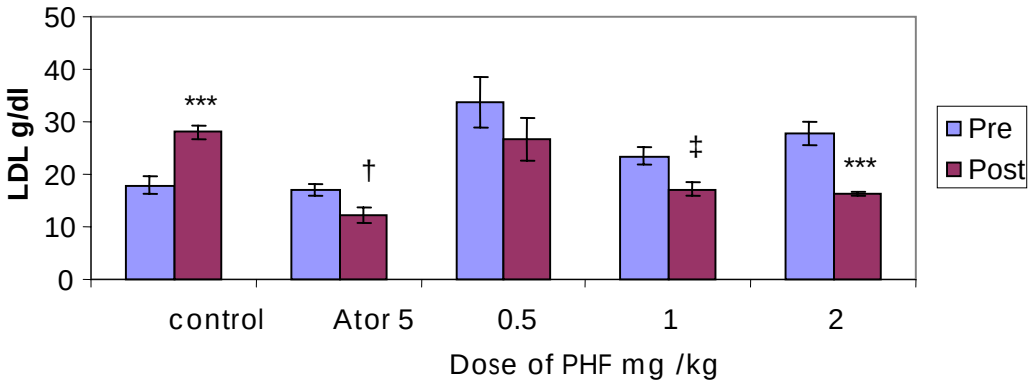
Fig 4 Effect of PHf and atorvastatin on LDL in rats fed with high cholesterol diet



Each value represents the mean ± SEM of six experiments
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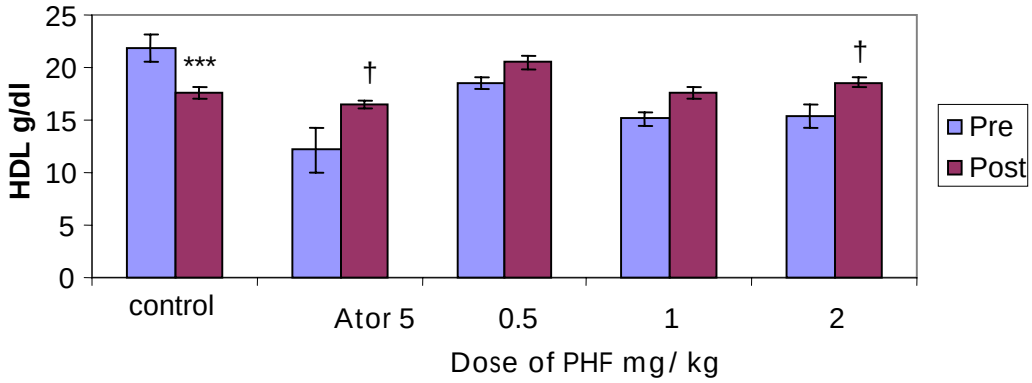
Fig 5 Effect of PHF and atorvastatin on HDL in rats fed with high cholesterol diet

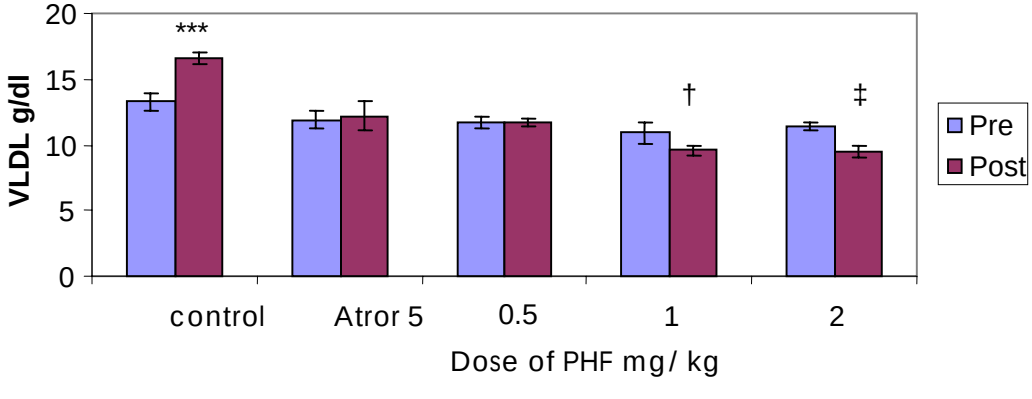


Each value represents the mean ± SEM of six experiments
†p <0.05, ‡p value<0.02 when compared with control value and ***p<0.01 when compared with the corresponding pre-treatment value

High Density Lipoprotein (HDL):

High cholesterol food decreased the HDL which was reversed by atorvastatin (36%). A similar observation was noticed in poly herbal formulation treated animals at all the doses (maximum 21%), however, the effect was not relating to the dose. (Figure: 5).

Fig 6 Effect of PHF and atorvastatin on VLDL in rats fed with high cholesterol diet



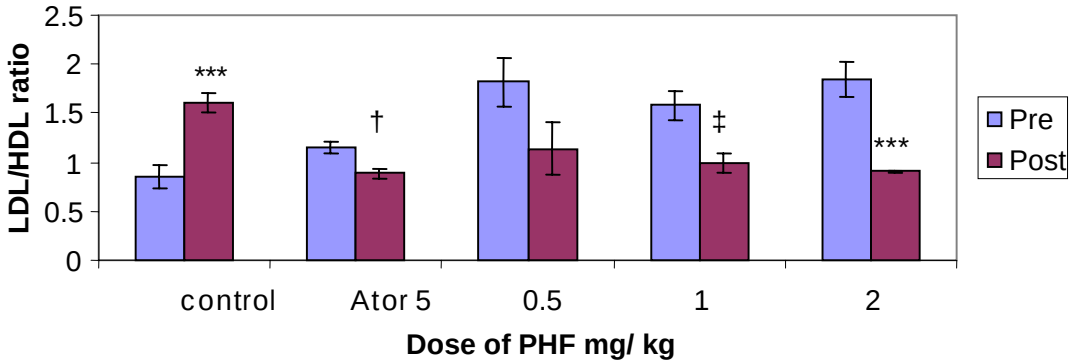
Each value represents the mean ± SEM of six experiments
†p <0.05, ‡p value<0.02 when compared with control value and ***p<0.01 when compared with the corresponding pre-treatment value

Very Low Density Lipoprotein (VLDL):

When the VLDL was measured, high cholesterol diet produced an increase, (25%) which was attenuated by atorvastatin (5%). In contrast, a dose related reduction in VLDL by poly herbal formulation (17% ; Figure 6) was noticed..

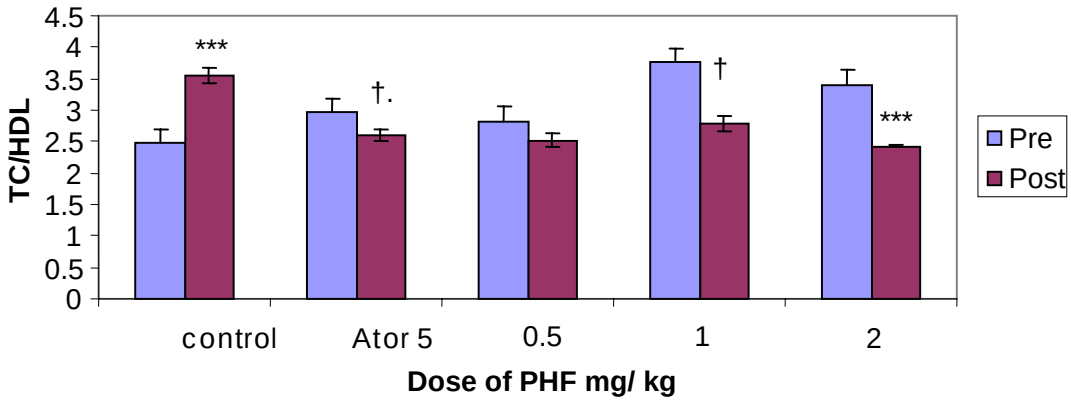
Based on the observed data, LDL/HDL, TC/HDL ratios were calculated using the formula described under materials and methods. In high cholesterol diet fed animals the ratios increased significantly (88%). In atorvastatin treated animals, a significant decrease in the ratios was recorded. A dose related decrease in the ratios (50 %) was observed in animals, which received different doses of poly herbal formulations (Figures: 7 & 8).

Fig. 7 Effect of PHF and atorvastatin on LDL/HDL ratio in rats fed with high cholesterol diet



Each value represents the mean ± SEM of six experiments
†p <0.05, ‡p value<0.02 when compared with control value and ***p<0.01 when compared with the corresponding pre-treatment value

Fig.8. Effect of PHF and atorvastatin on TC/HDL ratio in rats



Each value represents the mean ± SEM of six experiments
†p <0.05, ‡p value<0.02 when compared with control value and ***p<0.01 when compared with the corresponding pre-treatment value

Histopathological Examination:

The morphological observations revealed prominent fatty changes of hepatocytes with kuffer cell hyperplasia and diffuse fatty degeneration in high cholesterol diet fed animals. However, these changes were found to be minimal in atorvastatin as well as in poly herbal formulation treated animals. In aorta, high cholesterol diet induced diffuse sub minimal fatty changes with proliferation of tunica media. These changes were minimal in atorvastatin and poly herbal treated animals. In fact in 2g dose of poly herbal formulation treated animals, a normal histopathology of aorta was noticed. Apparently in all the groups, there was no significant change in myocardial cells.

Discussion

Hyperlipidemia induced atherosclerosis is known to induce coronary heart disease, ischemic cerebro vascular disease and peripheral vascular disease by altering the lipid profile (4). One way to manage without drug therapy is to reduce the obesity by diet restriction. (≤ 250-mg/ day) Statins are known to reduce the hyperlipidemia by inhibiting HMG COA reductase (4). In the present experimental study, atorvastatin justifies the model of high cholesterol diet used to identify hyperlipidemic effect.

As expected, atorvastatin reversed all the parameters prone for hyperlipidemia induced by high cholesterol diet. The identified poly herbal formulation also produced similar results on body weight, lipid profile, total reversal of the histo-pathological changes in the aorta produced by high cholesterol diet. These observations serve as scientific evidences for the folkloric claim of the ingredients of the formulation for its use in hyperlipidemia. This formulation has earlier been proved safe (11).

Therefore, it can be recommended that the use of this formulation as a food supplement may be beneficial in the management of hyperlipidemia. The possibility of regular chronic intake of this formulation in healthy individuals, as a prophylactic agent for preventing obesity and associated complications warrant additional experimentation, though acute toxicity conducted earlier prove to be safe.

References

1. Ganesh RS, Viswanathan P, Thirugnanasampantham P, Mayisvren, Anoop Austin G. A study on the effect of a herbal tea on elevated lipid profile in rats. International Journal Of Tropical Medicine. 2006; 1: 18-22.
2. Rastogi RP, Mehrotra BN. In; Compendium of Indian medicinal plants (CSIR) Publication of Informational Directorate, New Delhi. 1993.
3. Goldstein JL. Familial hypercholesterolemia. In; The Metabolic and Molecular Bases of Inherited Disease, 8th (ed), CR Scriver , New York, McGraw-Hill, 2001; pp. 2863-2913.
4. Maheley RW, Thomous P, Persort. The pharmacological basis of therapeutics. (Eds) Goodman and Gilman 10th edition. New york. Macgraw Hill medical publishing division; 2001; pp. 971-972.
5. Rubins HB. HDL Interventionl Study group. Amerian Journal of Cardiology. 1995; 75: 1196 - 1201.
6. Gale EAM, Anderson JV. Disorders of lipid metabolism, In ; clinical medicine. (Eds), Kumar and Clark. 2005; pp; 1135-1142.
7. Anoop Austin G, Senthilvel P, Thirugnanasampantham P, Mayisvren E. Clinical efficacy of a poly herbal instant formulation in the management of hyperlipidaemia, The Journal of Cardiology. 2006; 2: 36-38.
8. Flegg HM, Ann cain. In; Biochemistry. 1973; 10: 1350-1356.
9. Mc Grovan MW. Triglycerides (DES). In; Clinical chemistry. 1983; 29:538.
10. Friedwald WT. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of ultra-centrifuge. In; Clinical Chemistry. 1972; 18: 499-502.
11. Anoop Austin G, Jegadeesan M. Biochemical studies on the anti atherogenic potential of *Hemidesmus Indicus*. Journal of Ethnopharmacology. 2003; 84: 149-156.

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