Original Article

Evaluation of the anti-hyperlipidemic effects of *Triphala* in high fat diet fed rats: Studies with two combinations

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Abstract

Background: Hyperlipidemia is one of the major risk factors for coronary heart disease and stroke. *Triphala*, a polyherbal Ayurvedic formulation made from dried fruits of *Haritaki* (*Terminalia chebula* Retz.), *Bibhitaki* (*Terminalia bellirica* Roxb.), and *Amalaki* (*Phyllanthus emblica* Gaertn.) has been suggested to be useful in mitigating hyperlipidemia. In the Ayurveda texts, depending on the patient's condition and body type, *Triphala* is formulated and used in one of the two combination (1:1:1 and 1:2:4 of individual constituents) forms. **Aims:** The present study aimed at evaluating the efficacy of two combinations (1:1:1 and 1:2:4 of individual constituents) of *Triphala* against high fat diet induced-hyperlipidemia in rats. **Materials and methods:** Hyperlipidemia was induced in Spraque−Dawley albino rats by feeding them with high fat diet. The animals were concomitantly administered with graded dose of one of the two combination (combination of *Haritaki, Bibhitaki,* and *Amalaki* in ratio of 1:1:1 or 1:2:4, respectively) of *Triphala* (250, 500, or 1000 mg/kg body wt.) or atorvastatin. The animals were sacrificed on day 22 and serum was processed for lipid profile and the liver for lipid peroxidation. The statistical analysis was performed by the mean analysis of variance followed by Dunnet's test. **Results:** The results indicated that when compared to placebo group, levels of serum total cholesterol, and triglyceride were significantly lower, while high-density lipoprotein cholesterol increased in both the *Triphala* combination and atorvastatin groups. Of the two groups of *Triphala*, the formulation having 1:2:4 ratio was better than the 1:1:1. The group having highest drug dose (1000 mg/kg body wt.) of 1:2:4 formulation was better than atorvastatin in rectifying high fat diet-induced dyslipidemia and the atherogenic index was equal to that of atorvastatin. **Conclusions:** The results of the study indicate that of the two *Triphala* formulations, the 1:2:4 ratio was better than the 1:1:1 ratio for anti-hyper-lipidemic effects.

Keywords: Ayurveda, *Emblica officinalis* gaertn, hyperlipidemia, *Terminalia belerica* roxb, *Terminalia chebula* ratz, *Triphala*

Introduction

Hyperlipidemia, also known as hyperlipoproteinemia or high cholesterol, is caused by abnormal lipid and lipoprotein metabolism, which is a disorder characterized by abnormally high concentration of lipids (fats) in the blood that is correlated with the development of atherosclerosis, the underlying cause of coronary heart disease and stroke.[1] Lifestyle changes such as regular exercise, dietary modifications, abstinence from smoking, and excess alcohol consumption are suggested to be useful in the control of hyperlipidemia in early stages.[1] However, when lifestyle changes fail or the levels of serum cholesterol are in excess, the use of hypolipidemic drugs that affect the lipoprotein and cholesterol metabolism is recommended.[1] However, although useful, the regular use of hypolipidemic drugs is associated with untoward effects and may necessitate additional prophylaxis for the resulting side effects.

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Herbal medicines advocated and used in the Ayurvedic system of medicine are being appreciated for their beneficial effects and *Triphala*, a polyherbal formulation made from the dried fruits of *Haritaki* (*Terminalia chebula* Ratz.,

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Family-Combretaceae), *Bibhitaki* (*Terminalia bellirica* Roxb., Family-Combretaceae), and *Amalaki* (*Phyllanthus emblica* Linn. Family: Euphorbiaceae), is very useful.[2,3] In Ayurveda, *Triphala* is mentioned to be effective as a cardiotonic agent and previous studies have shown *Triphala* to possess an anti-hypercholesteremic effect in rats.[4] In a previous study, Wistar rats were fed with a high-fat diet (containing 4% cholesterol, 1% cholic acid, and egg yolk) for 48 days to increase the total cholesterol (TC), low-density lipoproteins (LDL), very-LDL, and free fatty acid. The concomitant administration of *Triphala* (1 g/kg body wt) caused a significant decrease in those parameters.[4]

In usual practice, *Triphala* formulation is made up of the three in equal proportion. However, a combination consisting of 1:2:4 of *Haritaki*, *Bibhitaki,* and *Amalaki*, respectively, is also recommended in Ayurveda.[2] The balance in *Tridosha* is highly sensitive and is influenced by age, environmental changes (diurnal, nocturnal, and seasonal modifications) and altered lifestyle (changes in food habits and physical activity).[2] The Ayurveda physicians recognized these aspects and placed importance to individualized treatment by prescribing medications with suitable drug combinations and ratio. *In lieu* of these observations and to validate the logic of Ayurveda physicians to be of selectively in using the two combinations, the present study was performed with both combinations of *Triphala* with a range of concentrations (125–1000 mg/kg) and including atorvastatin (10 mg/kg. wt) as a standard reference drug in high fat diet model in rats.

Materials and methods

Test drugs

The powders of fruits of *Terminalia bellerica* powders, *Terminalia chebula* and *Emblica officinalis* (batch no. AHTB-1102; AHTC-1106 and AHEO-1115, respectively), the individual constituents of *Triphala* were obtained from Amruta herbals, Indore, India. Atorvastatin (Atorlip 10) manufactured by Cipla batch no. D92544) was procured locally from a chemist; cholesterol from Spectrochem Pvt. Ltd., Mumbai and choline chloride from Spraque Dawley (SD) Fine Chemicals. The serum high-density lipoproteins (HDL) diagnostic kit, serum TC diagnostic kit, and serum triglyceride (TG) diagnostic kits used in the study were purchased from Swemed Diagnostics. All other reagents used were of analytical grade.

Preparation of extract

Triphala extract in 1:1:1 ratio (F1)

Equal quantities of extracts of fruit powder of *T. chebula*, *T. bellerica* and *E. officinalis* were taken and mixed using a blender/mixer for 5 min to attain uniform mixing.

Triphala extract in 1:2:4 ratios (F2)

Extracts of one part of *T. chebula*, two parts of *T. bellerica* and four parts of *E. officinalis* fruits were accurately weighed and mixed using a blender/mixer for 5 min to attain uniform mixing.

The aqueous extract of *Triphala* powder was prepared as described earlier.[3] Briefly, 100 g of the powder (1:1:1 or 1:2:4 ratio) was boiled in 1000 ml of deuterium depleted water till the volume was reduced to one fourth of the original (250 ml). The extract was then cooled and filtered in a two-step process. Initially, the extract was filtered using cotton cloth and then the supernatant collected was again filtered using two layers of filter paper. The filtrate obtained was then collected and concentrated by evaporating its liquid contents in china dish placed in a hot water bath maintained at 60°C. The extract obtained was scraped off from the china dish carefully and transferred in to a clean plastic bottle. An approximate yield of 22.5% and 28.7% of the extract was obtained for the 1:1:1 and 1:2:4 combinations of *Triphala,* respectively, and refrigerated until its further use.

Preparation of atorvastatin and *Triphala* extract for administration to rats

Atorvastatin at 10 mg/kg was dissolved in saline to get the working concentration.[5] The required amount of the drug (10 mg/Kg body weight) was freshly prepared in 2% Tween 80 before administering to the animals.[6] The preparation of the *Triphala* for administration and the dose of 1000 mg/kg body weight was selected from a previous study.[3,4] Briefly, appropriate amount of the *Triphala* extract (1000 mg) was accurately weighed and dissolved in sterile physiological saline on a magnetic stirrer. This was considered the stock drug and the lower strengths of 500 and 250 mg was obtained by appropriately diluting the stock (1:1 for 500 mg and 1:3 for 250 mg) with saline. The animals were orally administered 250, 500, or 1000 mg/kg body weight of CMC or *Triphala* extract through oral gavage.

Animal care

In-house laboratory bred healthy male SD rats, 10 weeks old and weighing 200–250 gm, were used for the experimental study. The animals were maintained under the standard conditions in an animal house approved by committee for the purpose of Control and Supervision of Experiments on animals. Three animals were housed per polypropylene cage under standard laboratory conditions at room temperature (25°C ± 2°C) with 12 h light/dark cycle. They had free access to standard pellet diet (M/s Gold Mohur Foods and Feeds Ltd., India) and water *ad libitum* except during experimentation. The study protocol was approved by the Institutional animal ethics committee (IAEC), St. John's Pharmacy College, Bangalore (proposal no. IJAHSM/IAEC/2008/009).

Preparation of high fat diet feed

The high fat diet was made in the laboratory by crushing the normally available rat feed first with a hand ball and then in an electrical blender. The required amount of the high fat constituent's 1% choline chloride, 2% cholesterol, and 2% lard[7] was added to the powdered normal diet and mixed thoroughly again in the blender to facilitate uniform mixing of the high fat ingredients. This mixture was then immediately mixed with equal quantity of water to obtain

semi solid consistent batter and then made into small pellets. The pellets were then dried in incubator maintained at 5°C. The resultant hard pellet was then stored in an air tight container and used as needed. High fat diet was made every week with utmost care adopting the standard laboratory practice and precautions to avoid fungal and vermin infestation. The food was fed to rats by placing them in the food holder on the cage.

Experiment 1: Standardization of the high fat diet-induced hyperlipidemia

Protocol standardization is a very important aspect in laboratory studies and to ascertain how hyperlipidemia progresses in rats, a pilot study was undertaken to observe for the optimal duration to study the high fat-induced hyperlipidemia. The high fat diet composed of 2% cholesterol, 1% choline chloride and 2% lard[7] prepared in the feed obtained from M/s Gold Mohur Foods and Feeds Ltd., India was provided to one group (n = 6) and nonhigh fat diet (normal diet; M/s Gold Mohur Foods and Feeds Ltd., India) to another (n = 6) in the feeding tray on the cage for 30 consecutive days in the food pellet form. Blood was collected before assorting the animals to two groups (start of experiment) and on every 7th day till the 28th day through the tail vein and evaluated for the serum TGs and TC values.

Experiment 2: Effect of Triphala in reducing the high fat diet (AD)-induced hyperlipidemia

Healthy male SD rats (n = 6) 10 weeks old and weighing 200–250 g, were selected and assigned to the following groups. Group 1: Control group treated with saline solution; Group 2: High fat diet only (no treatment given); Group 3: High fat diet + F1 (125 mg/kg bw); Group 4: High fat diet + F1 (250 mg/kg bw); Group 5: High fat diet + F1 (500 mg/kg bw); Group 6: High fat diet + *Triphala* F1 (1000 mg/kg bw); Group 7: High fat diet + *Triphala* F2 (125 mg/kg bw); Group 8: High fat diet + *Triphala* F2 (250 mg/kg bw); Group 9: High fat diet + F2 (500 mg/kg bw); Group 10: High fat diet + *Triphala* F2 (1000 mg/kg bw); Group 11: High fat diet + Atorvastatin at 10 mg/kg.

Care was taken to see that the cages and the animals of each group were kept in different corners of the room in animal house in appropriately labeled steel racks and cages. Care was also taken to see that the extracts of F1 or F2 extract or atorvastatin did not get mixed up by taking up one extract/drug at a time and double checking the animals group with the steel rack, animal cage, the animal body markings with the dose of extract to be given. The second group was taken up only after the completion of the first group by the student investigator under the supervision of the mentor.

Animal sacrifice and collection of blood and liver

On day 22, the animals were fasted overnight (evening 8 PM to morning 8.00 AM) in a base meshed cage (to avoid cacophagia) with access only to water. They were anesthetized with diethyl ether and blood was collected by retro orbital puncture in to a clean centrifuge tube and allowed to clot for 30 min at room temperature.[8] Following this, the rats were humanely euthanized with over dose of ether, dissected and the liver were collected after transcardial perfusion with sterile ice cold saline. One lobe of the liver was removed, blot dried, weighed, and processed for lipid peroxidation assay as described by Ohkawa *et al*.[9]

Biochemical estimations

The tube was then subjected to centrifugation at 2000 rpm for 15 min and the upper serum layer was collected in a fresh prelabeled micro-centrifuge tubes. The serum was stored in refrigeration and analyzed for serum TGs, TC and HDL using the respective kits accordance to the instruction manual in an autoanalyzer (Brand model). The atherogenic index (AI) was calculated using the formula AI = (TC-HDL-C)/HDL-C;[10] TC: HDL-C ratio using the formula TC/HDL-C[11] and Non HDL-C using the formula TC-HDL-C.[11] The liver collected was immediately weighed and the 10% homogenate was prepared with PBS. The levels of lipid peroxidation (as thiobarbituric acid [TBA] reactive substances) and protein in liver was estimated using the standard methods of Ohkawa *et al*.[9] and Lowry *et al*.,[10] respectively. The resultant concentration of TBA reactive substances was expressed as nmol/mg protein obtained from a standard curve of tetraethoxypropane.

Statistical analysis

The results were expressed as mean ± S. E. M (n = 6). The statistical analysis involving eleven groups was performed by means analysis of variance followed by Dunnett test. P <0.05 was considered as statistically significant. Data were analyzed with graph pad prism version 5.00 software.

Results

Standardization of high fat diet-induced hyperlipidemia in Sprague Dawley albino

In the preliminary study, it was observed that feeding Sprague Dawley albino rats with high fat diet consisting of 2% cholesterol, 1% choline chloride and 2% Lard for 30 days induced hyperlipidemia, and the effect was time dependent. [Figure 1a and b] When compared to the normal pellet diet fed cohorts, feeding high fat diet did not caused a significant increase in serum lipid profile on day 7 and 14. Significant increase in the levels of TC (2.1 folds) [Figure 1a] and TG (2.2 folds) [Figure 1b] were seen on day 21 post-initiation. A further increase in the feeding of the high fat diet to 28 days did not increased in the levels of TC and TG (when compared to day 21). Together all these observations suggest that feeding high fat diet for 21 days was sufficient to cause hyperlipidemia in Sprague Dawley albino rats and was used for further studies.

Effect of treatment on serum parameters

The values obtained for the different serum parameters tested in the present study are given in Table 1. It was observed that

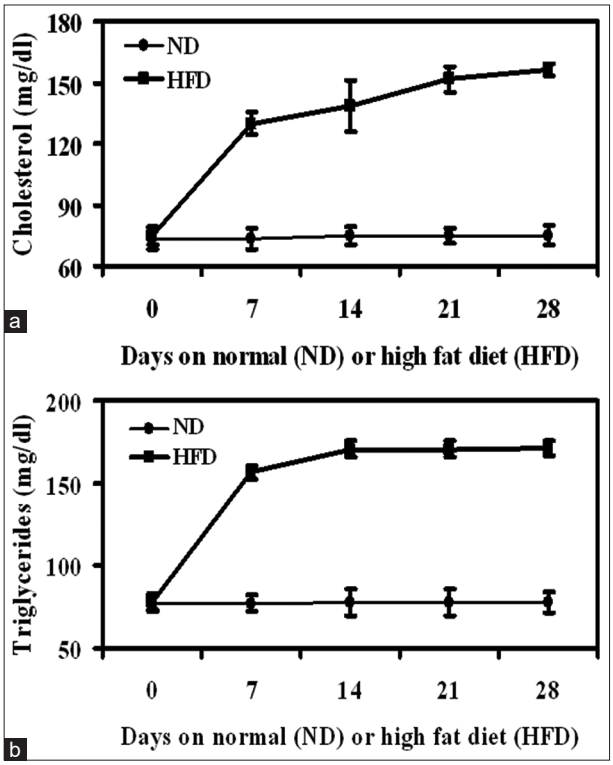

Figure 1: Effect of HFD feeding for 21 days on the lipid profile in SD rats (a) Total cholesterol; (b) triglycerides. ND: Normal diet; HFD: High fat diet

when compared to the normal diet, feeding rats with high fat diet altered the levels of lipid profile and increased the atherogenic index (AI) significantly. [Table 1; details of column 2 vs. 3] Feeding rats with high fat diet cause alterations as follows for TC (68.8 ± 4.2 vs. 144.8 ± 7.7; a fold increase of 2.1 and P = <0.001); TG (81.1 ± 8.6 vs. 174.9 ± 4.5; a fold increase of 2.15 and P = <0.001); HDL (39.6 ± 3.2 vs. 30.3 ± 1.8; fold decrease of 0.76 and P = <0.01); non HDL (29.2 ± 2.8 vs. 114.5 ± 8.3; a fold increase of 3.92 and P = <0.001) and in the TC: HDL ratio (1.8 ± 0.1 vs. 4.9 ± 0.4; fold increase of 2.72 and P = <0.001). The results also showed that feeding rats with high fat diet increased the AI (0.8 ± 0.1 vs. 3.9 ± 0.4; fold increase of 4.87 and P = <0.001).

Administration of atorvastatin to the high fat diet fed rats reversed the changes and was significant. [Table 1; Column 2 vs. 3] Administration of atorvastatin to the high fat diet fed rats caused these alterations in TC (144.8 ± 7.7 vs. 73.2 ± 6.9; fold decrease 0.50 and P < 0.001); TG (174.9 ± 4.5 vs. 103.6 ± 4.9; fold decrease of 0.59 and P < 0.001); HDL (30.3 ± 1.8 vs. 35.6 ± 2.9; fold increase of 1.17 and P < 0.05); non HDL (114.5 ± 8.3 vs. 37.6 ± 5.5; fold decrease of 0.32 and P < 0.001) and in the TC: HDL ratio (4.9 ± 0.4 vs. 2.1 ± 0.1; fold decrease of 0.43 and P < 0.001). Administration of atorvastatin resulted in almost three-fold decrease in the AI (3.9 ± 0.4 vs. 1.0 ± 0.1; P < 0.001).

Administration of *Triphala* in both F1 and F2 combinations to the high fat diet fed rats caused dose dependent alterations in the lipid profiles and decrease in the AI. [Table 1] Both

Table 1: Effect of two combinations of *Triphala* on high fat diet induced changes in serum lipid levels

Para-meters	Normal control	AD diet control	Atorvastatin	AD+F1				AD+F2			
				125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
TC[f]	68.8±4.2	144.8±7.7[c]	73.2±6.9[r]	114.6±6.3[b,@]	100.9±13.9[p]	94.9±2.0[q]	89.0±5.0[q]	110.5±19.7[a]	92.7±7.8[q]	85.9±6.7[r]	75.1±7.1[r]
TG[f]	81.1±8.6	174.9±4.5[c]	103.6±4.9[r]	140.7±8.5[c]	136.1±11.5[b]	131.7±9.5[b,p]	122.0±4.7[a,q]	138±10.5[b]	126.8±14.3[a,p]	118±13.5[q]	106.8±11.1[r]
HDL[f]	39.6±3.2	30.3±1.8[b]	35.6±2.9	29.7±1.3[b]	30.6±1.1[a]	32.9±1.3	32.0±1.7[a]	31.9±1.0[a]	33.6±2.0	34.2±1.2	37.3±1.9[p]
Non-HDL[f]	29.2±2.8	114.5±8.3[c]	37.6±5.5[r]	84.9±7.1[c,#]	70.3±12.9[a,p]	61.9±2.4[q]	57.0±5.7[r]	78.6±18.9[b,@]	59.1±8.2[q]	51.8±7.2[r]	37.7±7.5[r]
TC HDL	1.8±0.1	4.9±0.4[c]	2.1±0.1[r]	3.9±0.4[c,#]	3.3±0.4[b,q]	2.9±0.1[r]	2.8±0.3[r]	3.4±0.5[b,p,@]	2.8±0.3[r]	2.5±0.2[r]	2.0±0.2[r]
AI[Δ]	0.8±0.1	3.9±0.4[c]	1.0±0.1[r]	2.9±0.4[c,#]	2.3±0.4[b,q]	1.9±0.4[r]	1.9±0.1[r]	2.4±0.5[b,p,@]	1.8±0.3[r]	1.5±0.2[r]	1.0±0.2[r]

F1=1:1:1 of *T. chebula*, *T. bellerica* and *E. officinalis* and F2=1:2:4 of *T. chebula*, *T. bellerica* and *E. officinalis*. Values are mean±SEM (n=6). [f]mg/dL, [Δ]units. The analysis of the data was performed with three principal criteria's for consideration. The first being comparison of the various treatment groups with normal diet fed rats (normal control) and the statistical values represented with superscripts of the alphabets as "[a]<0.05, [b]<0.01 and [c]<0.001" in Table 1. Second was comparison of various treatments (atorvastatin and *Triphala*) with the high fat diet and the statistical values represented with superscripts of the alphabets as "[p]<0.05, [q]<0.01, [r]<0.001" in Table 1. Finally the comparison of various doses of *Triphala* combinations with Atorvastatin and the statistical values represented with superscripts of symbols [@]<0.05, [#]<0.01, *<0.001 in the Table 1. *P*: [a]<0.05, [b]<0.01, <0.001, as compared with normal control; [p]<0.05, [q]<0.01, [r]<0.001, as compared with AD diet. HDL: High-density lipoproteins, TC: Total cholesterol, TG: Triglyceride, AI: Atherogenic index, ANOVA: Analysis of variance, SEM: Standard error of mean, *T. chebula: Terminalia chebula, T. bellerica: Terminalia bellirica, E. officinalis: Emblica officinalis*, AD: Ayurvedic diet

Triphala F1 and F2 had a profound effect in reversing the high fat induced dyslipidemia and the effects were comparable to that of 10 mg/kg b wt. atorvastatin. For clarity in understanding the data comparing the protective effects of two combinations of *Triphala* and atorvastatin are expressed in percentile grade changes. [Table 1]

The results when considered along with the percent changes clearly indicated that at equivalent concentrations, the F2 combination was better than F1. Further it was also observed that the highest drug dose of 1000 mg/kg b. wt. was better than even atorvastatin in rectifying the high fat diet induced dyslipidemia in the levels of HDL (atorvastatin 35.6 ± 2.9 vs. F2 37.3 ± 1.9) and that the AI was equal to that of the cohort that received atorvastatin. [Table 1, Figure 2]

Effect of treatments on liver lipid peroxidation

Compared to normal control group, rats fed with high fat diet had highly significant increase in liver lipid peroxidation (LPO) levels ($P < 0.001$). In comparison to high fat diet control group *Triphala* (500 mg/kg, po) of F1 and *Triphala* (250 mg/kg, po) of F2 significantly decreased the LPO levels ($P < 0.05$) in rats, whereas *Triphala* (1000 mg/kg, po) of F1 and *Triphala* (500 mg/kg, po) of F2 had shown high significance in reducing LPO levels ($P < 0.01$) to normal. *Triphala* (1000 mg/kg, po) in F2 showed the highest significance in reducing the LPO levels ($P < 0.001$) in rats. When compared to positive control of Atorvastatin. *Triphala* (125 mg/kg, po) and *Triphala* (250 mg/Kg, po) of F1 had shown highly significant difference in LPO ($P < 0.001$)

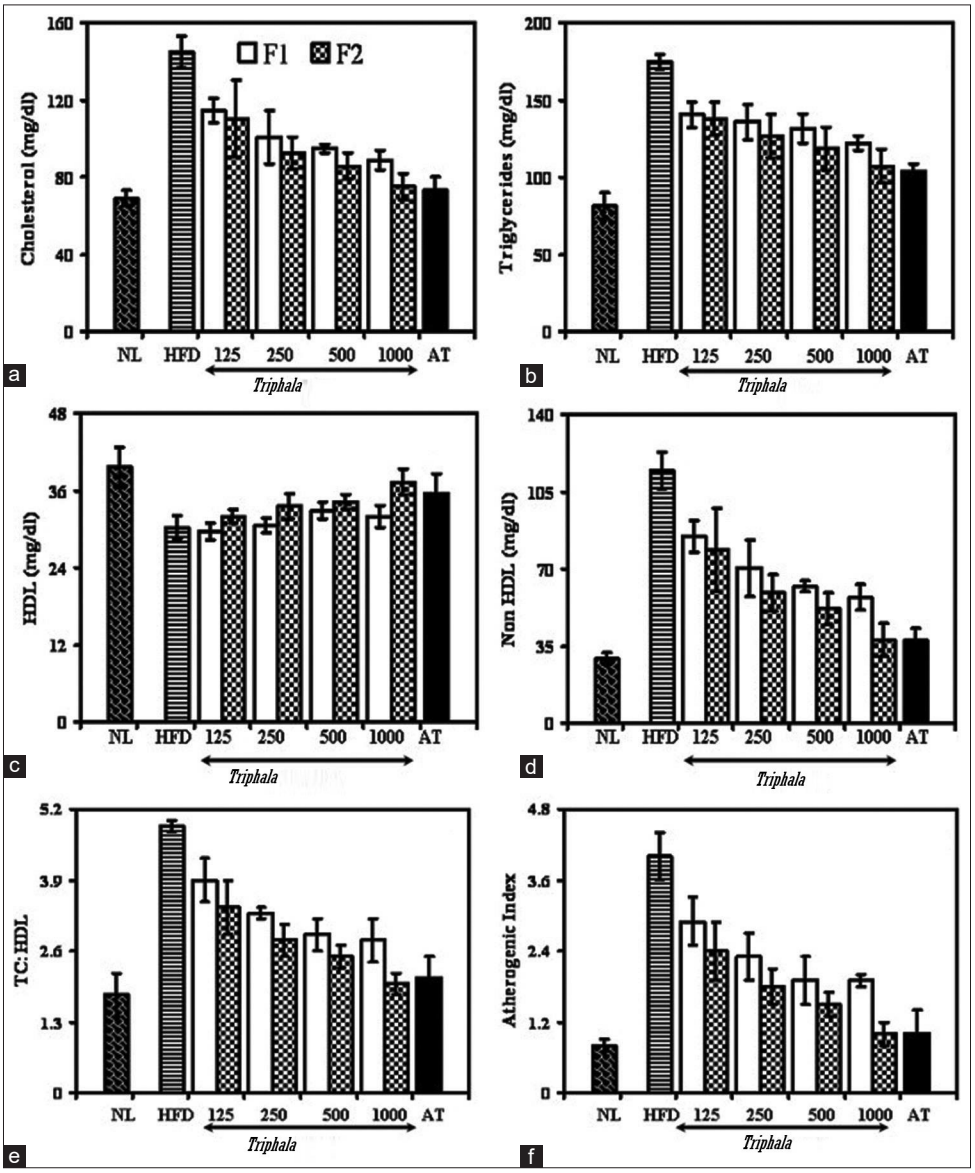

Figure 2: Effect of *Triphala* on HFD + F1 and high fat diet + F2 and Atorvastatin (10 mg/kg, p.o.) on serum lipid levels ([a] total cholesterol; [b] triglycerides; [c] HDL; [d] Non HDL; [e] Total cholesterol: HDL ratio and [f] atherogenic index). F 1 = *Triphala* made of 1:1:1 of *Haritaki*, *Bibhitaki* and *Amalaki*. F 2 = *Triphala* made of 1:2:4 of *Haritaki*, *Bibhitaki* and *Amalaki* AT: AtorvastatinNL: Rats fed normal diet. These were not fer high fat diet or *Triphala* or Atorvastatin. HFD: High fat diet

whereas *Triphala* (500 mg/kg, p. o) of F1 and *Triphala* (250 mg/kg) of F2 showed decrease in LPO ($P < 0.01$) and ($P < 0.05$) respectively, which means they have not reduced LPO levels significantly as compared to atorvastatin. [Figure 3]

Discussion

Nutrition is known to play a cardinal role in hyperlipidemia and the experimental model of feeding rats with high fat diet, adopted for the present study is globally accepted as it closely resembles the human pathogenesis.[6,8] Sprague Dawley albino rats were used, as this strain of rats are better model than the Wistar rats for hyperlipidemic studies.[7] In this model, in addition to cholesterol, high levels of saturated fatty acids and cholic acid salts are incorporated to amplify the effect of dietary cholesterol and result in hypercholesterolemia.[11-13] In this study it was observed that feeding rats with a high fat diet increased the TC, TG, non-HDL, TC: HDL and AI, and also that the administration of atorvastatin reduced these levels. These observations are in agreement to the earlier reports and validate this experimental model in our laboratory conditions.[14-16]

In this study it was observed that both F1 and F2 combinations of *Triphala* were effective in reducing the high fat diet-induced hyperlipidemia. A concentration dependent decrease in the levels of TC, TG, non-HDL, TC: HDL and AI, and a concomitant increase in the levels of HDL were observed. Our observations with the F1 are in agreement to the earlier observations[4] clearly indicating the usefulness of *Triphala* as a hypolipidemic agent. Of the two combinations, the F2 was observed to be better than F1 as at all concentrations the protective effects were more prominent. Optimal protective effects were observed at 1000 mg/kg body weight with F2 combination, where most of the results were almost equal to that of atorvastatin. Our ongoing studies have also shown that the F2 combination of *Triphala* contains proportionately more amounts of vitamin C and polyphenols than the F1 combination and this may be the reason for the difference in the protective effects (unpublished observations).

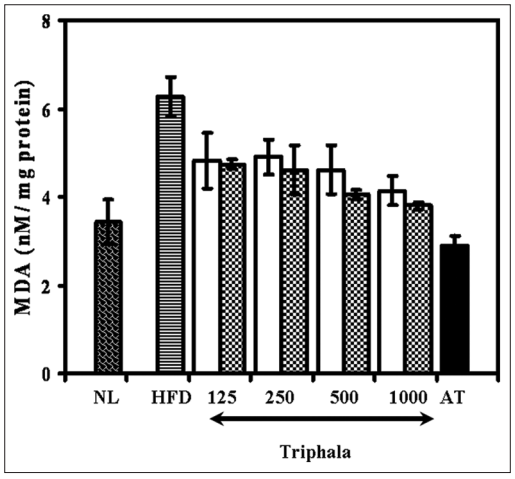

Figure 3: Effect of *Triphala* in preventing the high fat diet induced lipid peroxidation in the liver. AT: Atorvastatin NL: Rats fed normal diet. These were not fer high fat diet or *Triphala* or Atorvastatin. HFD: High fat diet

When compared to the F1 combination that contains the three constituents in equal ratio, F2 is made of one part of *T. chebula*, two parts of *T. bellerica* and four parts of *E. officinalis*[17] and the difference in the hypolipidemic effects may be due to this. Earlier studies have also shown that the F2 formulation was more effective than the F1 in protecting against the methotrexate-induced enterotoxicity[17] indicating its beneficial effects. The difference in the hypolipidemic effects may lie in the combination of the three myrolabans in the two *Triphala* combinations.

With respect to the individual constituents, earlier studies have conclusively shown that *T. chebula*,[14,15] *T. bellerica*[14-16] and *E. officinalis*[14,18-25] possess hypolipidemic effects in laboratory animals. Additionally, recent studies by Makihara *et al*.[26] have also shown that administering *T. bellirica* was effective in preventing obesity and metabolic disorders in spontaneously obese type 2 diabetic model mice. *Triphala* has been shown to scavenge free radicals,[3,27-30] possess anti-inflammatory effects,[31,32] immunomodulatory effects,[33-35] decreases lipid peroxidation,[27,28,34-36] and to increases the levels of antioxidants and antioxidant enzymes;[36] all of which contributes to the beneficial effects. In addition to these mechanisms it is quite possible that *Triphala* may mediate the hypolipidemic action by: (a) decreasing cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, (b) increasing degradation of cholesterol to fecal bile acids and neutral sterols, (c) increasing lecithin–cholesterol acyltransferase (LCAT) activity; while the observed hypotriglyceridemic effect may be due to (a) decreased fatty acid synthesis, (b) increased lipolytic activity by inhibiting hormone-sensitive tissue lipases or suppression of lipogenic enzymes and (c) activation of LCAT and tissues lipases. Mechanistic studies are required to validate the effect of *Triphala* on these targets.

Way forward

The results of this study indicate that the ratio of the formulation has an important role in inducing the effectiveness. Validation of *Tridosha* in experimental animals is not feasible and clinical studies are underway to validate the usefulness of the type of *Triphala* to be used as per the Ayurvedic concepts and the mechanism/s responsible for the effect. From the perceptiveness of extrapolation of dose for animals the maximum dose of 1000 mg/kg body weight used amounts to 11.2 g, pilot studies should be undertaken to understand the maximum permissible dose in humans and the outcomes of these studies will be useful. However, present preclinical study has confirmed that *Triphala* has a high margin of drug safety and needs to be studied in detail in people affected with hyperlipidemia.

Conclusions

The results of the study provided convincing evidence for the beneficial effects of *Triphala* in reducing the high fat

diet-induced hyperlipidemia and levels of lipid peroxides. Of the two formulations the 1:2:4 ratio formulation was observed to be better than the 1:1:1 ratio formulation in offering better protection and was equal to the protective effects of atorvastatin used as the standard drug. The difference in the hypolipidemic effects may lie in the combination of the three fruits in the two *Triphala* combinations. The greater proportion of *Amlaki* in the 1:2:4 combinations may have enhanced the protective effects.

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Conflicts of interest

There are no conflicts of interest.

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