—Regular Article—

Hypolipidemic Effect of Arborium Plus in Experimentally Induced Hypercholestermic Rabbits

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Hypercholesteremia is one of the risk factors for coronary artery disease. The present study highlights the efficacy of the ayurvedic herbal formulation Arborium Plus [*Hyppophae ramnoides* L. fruit juice (S) and *Rhododendron arboreum* Sm. Linn flower juice (R) in a 1 : 4 ratio] on triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), atherogenic index (AI), high-density lipoprotein (HDL), and high-sensitivity c-reactive protein (hs CRP) in experimentally induced hypercholesterolemic rabbits. Four groups of rabbits were subjected to different treatments for 8 weeks: control group, CHOL group (1% w/w cholesterol for 8 weeks), S+R group (1% w/w cholesterol and Arborium Plus for 8 weeks), and A group (1% w/w cholesterol and atorvastatin for 8 weeks). The results showed significant increases in TG, TC, LDL, AI, and hs CRP in hypercholesterolemic rabbits which was significantly reduced in Arborium Plus-treated hypercholesterolemic rabbits. The data demonstrated that the Arborium Plus formulation was associated with hypolipidemic effects in experimentally induced hypercholesterolemic rabbits.

Key words——hyperlipidemia; Oil Red O; sea buckthorn; Rhododendron; atherogenic index; c-reactive protein

INTRODUCTION

Coronary artery disease (CAD) is one of the most important causes of death all over the world.[1,2] Hypercholesterolemia is one of the risk factors for CAD. Increased serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) as well as lowered levels of high-density lipoprotein (HDL) and increased levels of hs c-reactive protein (CRP, a nonspecific inflammatory protein) have been identified in the development of hypercholesterolemia.[3] Those with a combination of risk factors (dietary habits and genetic susceptibility) are more prone to develop hypercholesterolemia. In addition to stress and sedentary habits, the use of alcohol and tobacco are reported to have a cumulative effect in contributing to the development of hypercholesterolemia.[4] The initial step in reversing the progression of hypercholesterolemia is the modification of the nutritional regimen with a diet low in fats and saturated fatty acids and rich in crude fibers.

Herbs in the Indian system of medicine have been reported to be beneficial in treating hypercholesterolemia.[5,6] *Hyppophae ramnoides* L. (sea buckthorn), commonly known as sallow thorn is rich in ascorbic acid, tocopherols, carotenoids and polyphenols in its seeds, fruit and leaves. Its high nutritional value has long been recognized and recently several studies have confirmed its lipid-lowering, antioxidant, antiplatelet-activities.[7–13] *Rhododendron arboreum* Sm. is commonly known as buruns in the Himalayas and is rich in polyphenols, flavonoids, and vitamin P. Several studies provided evidence for its antiinflammatory properties and strong vitamin P-like activity that protects against capillary fragility. Based on the above, the present study was designed to evaluate the combined effects of these two herbs on hyperlipidemia and associated vascular events.[14–16] Arborium Plus is a herbal combination of *H. ramnoides* L. fruit juice and *R. arboreum* Sm. flower juice in a 1 : 4 ratio formulated by Redhill Herbal Ltd. Several synthetic drugs presently available for treating hyperlipidemia have serious side effects, and there is a need to develop better drugs for treating the condition and associated vascular changes. *H. ramnoides* L. and *R. arboreum* Sm. are time-tested ayurvedic natural herbs with medicinally valid active principles like flavones, polyphenols, vitamin C, vitamin P, tocopherols, and essential minerals that together may be of great im-

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portance in treating hyperlipidemia, inflammation, thrombosis, platelet aggregation, and capillary fragility. The present study was undertaken to identify the benefits of the herbal combination on hyperlipidemia and associated vascular changes by comparing it with the synthetic HMG-CoA reductase inhibitor atorvastatin.[17–19]

MATERIALS AND METHODS

Herb authentication tests were done at the National Institute of Science Communication and Information Resources, New Delhi (NISCAIR/RHM/F-3/Conslt /06/690/07).

Drug and Dosage Arborium Plus (equivalent to 300 mg/kg/d) was administered using an oral infant feeding tube inserted through a wooden oral gag. Atorvastatin (5 mg/kg/d) was administered as a suspension prepared using 0.5% carboxymethyl cellulose (CMC).

Animals One-month-old New Zealand white rabbits (1000–15000 g) of either sex were selected for this study and used with the approval of the Institutional Animal Ethics Committee. They were housed in a well-ventilated animal unit with normal daylight (12-h light/dark cycle, lights on at 07:00). The animals were fed with a standard (Amrit Laboratory Feed Pvt. Ltd., India) rabbit chow diet and water *ad libitum*. The animals were divided into four groups ($n=6$), and the following treatments were given simultaneously to each group for 8 weeks.

Experimental Groups The control group received the standard chow diet and water. In the treatment groups, the CHOL group was fed a f-enriched (1% w/w cholesterol+10% v/w groundnut oil) diet, the S+R group was fed a f-enriched (1% w/w cholesterol+10% v/w groundnut oil) diet plus test drug (Arborium Plus 300 mg/kg/d), and the A group was fed a f-enriched (1% w/w cholesterol+10 % v/w groundnut oil) diet plus the standard drug (atorvastation 5 mg/kg/d).[20,21]

Estimation of Biochemical Parameters After the experimental regimen, the animals were subjected to overnight fasting, although water was provided *ad libitum*. Two-milliliter blood samples were drawn using a marginal ear vein puncture and centrifuged. The serum was then collected and used for biochemical measurements. Biochemical parameters were determined using the following kits. LDL cholesterol, TC, TG, and HDL cholesterol kits were procured from Qualigens Diagnostics, and the hs CRP kit was A Spinreact from ARK Diagnostics. Heparin sodium (Beparin) was procured from Biological E. Limited, India. The cholesterol powder was procured from SD Fine-Chem Limited, India.

Qualitative Differences in Aortal Strips Gross morphologic changes in the endothelium of the aortal strips are determined in separate animals on an identical feeding schedule. Quality was determined based on a comparison among aortal specimens of staining degree and thickness.[22] Animals were anesthetized with an overdose of pentobarbital. The right carotid artery, femoral artery, and vein were cannulated. Shortly before euthanasia, beparine (heparine, 5000 IU units) was injected intravenously through the marginal ear vein to prevent intravascular blood coagulation. The arterial system was perfused immediately with heparinized saline (2 U/ml) *via* the right femoral artery at a pressure of 120 mmHg. After the fluid drained from the vein became clear, the perfusate was switched to a neutral-buffered 10% formalin solution. Perfusion was continued at the same pressure for another 10 min as a preliminary perfusion fixation. For further fixation, the thoracic aorta was excised and immersed in 10% formalin for 1 h. The advential tissue and branches were carefully removed. Each aortic specimen was cut longitudinally and opened inside out. The specimens were stained with Oil Red O.

Oil Red O Preparation A small amount of propylene glycol was added to Oil Red O (0.5 g) and mixed well, crushing the larger pieces with a stirring bar. The remaining propylene glycol was added gradually while stirring. The solution was heated gently until it reached 100℃, then filtered through coarse (25 μm) filter paper while still warm. The solution was left overnight at room temperature and used directly for staining.[23]

Statistical Analysis Data are presented as mean ±S.D. of 6 animals. Statistical evaluation was done using one-way analysis of variance (ANOVA) with SPSS version 11.5. For comparison between the groups, the significance level was fixed at $p<0.001$.

RESULTS

Biochemical Parameters

Changes in Serum TC Cholesterol feeding induced a significant increase in serum TC ($p<0.001$) as compared with the control group (1905±95.7 *vs.*

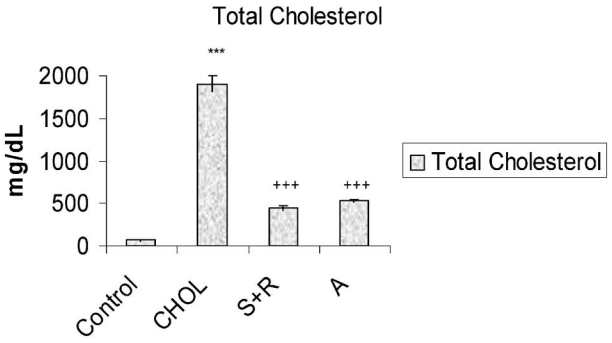

Fig. 1. Serum Total Cholesterol in Different Groups after 8 Weeks
($n=6$), $^{***}p<0.001$ *vs.* control; $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

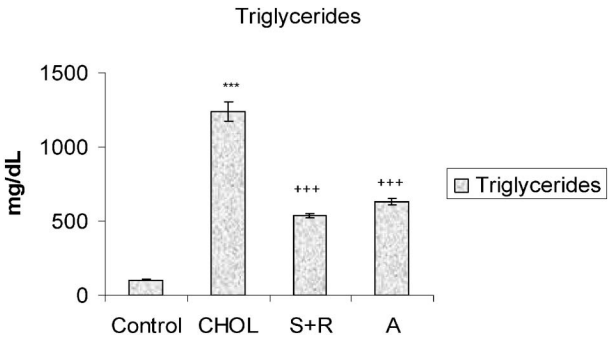

Fig. 2. Serum Triglycerides in Different Groups after 8 Weeks
($n=6$), $^{***}p<0.001$ *vs.* control; $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

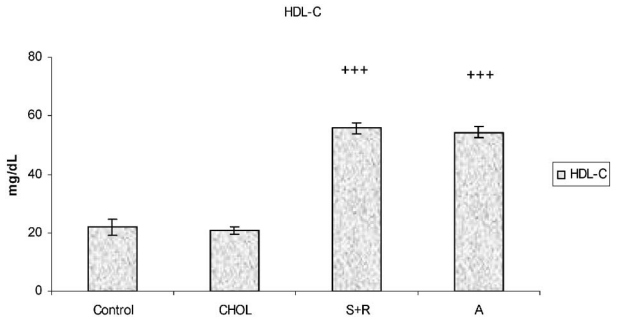

Fig. 3. Serum HDL-C in Different Groups after 8 Weeks
($n=6$), $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

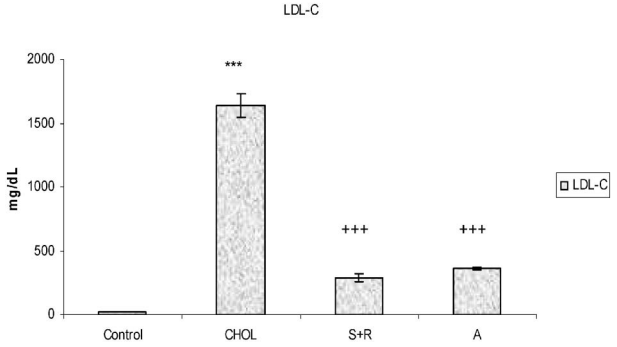

Fig. 4. Serum LDL-C in Different Groups after 8 Weeks
($n=6$), $^{***}p<0.001$ *vs.* control; $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

64.83±1.67 respectively) (Fig. 1). Arborium Plus and atorvastatin treatment prevented the increase in serum TC ($p<0.001$) as compared with the CHOL group (449.8±29.9, 537.7±13.3 *vs.* 1905±95.7 respectively).

Changes in Serum TG Cholesterol feeding significantly increased serum TG ($p<0.001$) as compared with the control group (1240±65 *vs.* 103±2.45 respectively) (Fig. 2). Arborium Plus and atorvastatin treatment significantly prevented the increase in serum TG ($p<0.001$) as compared with the CHOL group (534±15.9, 630±23.5 *vs.* 1240±65 respectively).

Changes in Serum HDL Cholesterol Serum HDL cholesterol was lower, although not significantly, in the CHOL group and was significantly ($p<0.001$) higher in the S+R group and A group, when compared with the control group (20.71±1.31, 55.78±1.86, 54.37±1.97 *vs.* 21.89±2.77 respectively) (Fig. 3). In the S+R and A groups, serum HDL was significantly ($p<0.001$) higher than in the CHOL group (55.78±1.86, 54.37±1.97 *vs.* 20.71±1.31 respectively).

Changes in Serum LDL Cholesterol Cholesterol feeding induced a significant increase in serum LDL cholesterol ($p<0.001$) as compared with the control group (1637±92.8 *vs.* 22.33±2.8 respectively) (Fig. 4). Arborium Plus and atorvastatin treatment prevented the increase in serum LDL cholesterol ($p<0.001$) as compared with the CHOL group (287.2±29.57, 357.3±10.46 *vs.* 1637±92.8 respectively).

Changes in Serum AI Cholesterol feeding induced a significant increase in the AI ($p<0.001$) as compared with the control group (91.55±2.67 *vs.* 2.07±0.27 respectively) (Fig. 5). Arborium Plus and atorvastatin treatment prevented the increase in the AI ($p<0.001$) as compared with the CHOL group (7.13±0.65, 8.96±0.42 *vs.* 91.55±2.67).

Changes in Serum CRP Cholesterol feeding induced a significant increase in serum CRP ($p<0.001$) as compared with the control group (5.14±0.55 *vs.*

0.11±0.032 respectively) (Fig. 6). Arborium Plus and atorvastatin treatment significantly prevented the increase in serum LDL cholesterol ($p<0.001$) as compared with the CHOL group (0.86±0.13, 0.67±0.15 vs. 5.14±0.55 respectively).

Gross Morphology Qualitative examination of Oil Red O-stained rabbit aortic strips revealed intense red coloration in the CHOL group as compared with the control group indicating the development of fatty streaks (Fig. 7). The aortas of animals treated with arborium or atorvastatin showed significantly less red coloration.

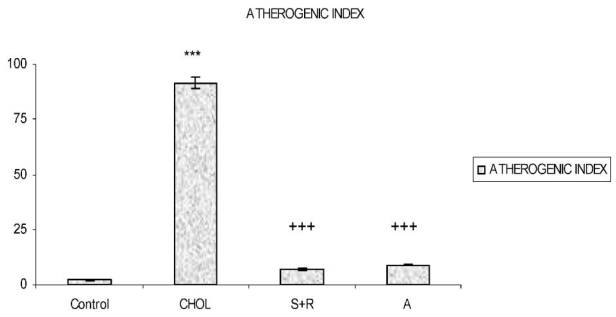

Fig. 5. Atherogenic Index in Different Groups after 8 Weeks
($n=6$), $^{***}p<0.001$ *vs.* control; $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

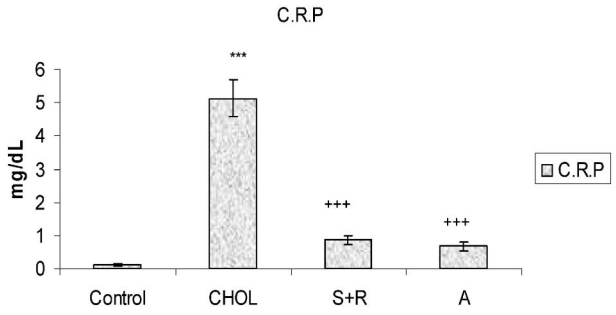

Fig. 6. Serum CRP in Different Groups after 8 Weeks
($n=6$), $^{***}p<0.001$ *vs.* control; $^{+++}p<0.001$ *vs.* group CHOL. Values are expressed as mean±S.E.M.

DISCUSSION

The present study revealed the hypolipidemic activity of Arborium Plus in experimentally induced hypercholesterolemia in rabbits. Cholesterol-fed rabbits are often used as a model to reflect human conditions.[24] In this model, a high-fat diet was used to produce hyperlipidemia and the desired lesions in the arterial endothelium consistently. In the present study, a cholesterol-enriched diet with groundnut oil supplementation resulted in hyperlipidemia in rabbits. Rabbits fed with cholesterol and groundnut oil showed significant increases in TG, TC, and LDL cholesterol, confirming that cholesterol feeding (1% cholesterol+10% groundnut oil) induced hypercholesterolemia in this study. With reference to the toxicity studies conducted (Shriram Institute for Industrial Research, job No. 305-451-0144, annexure to report No. 000057715), the significant reduction in TG, TC, LDL cholesterol, and hs CRP is not related to any overt toxicity.

The present study showed that the administration of Arborium Plus (300 mg/kg/d) significantly reduced TG, TC, and LDL cholesterol, while elevating HDL cholesterol when compared with the cholesterol-fed group. In addition, Arborium Plus reduced the AI *i.e.*, total cholesterol to HDL cholesterol ratio, indicating a reduction in the harmful lipid pool relative to the favorable one. Moreover, the results indicated that there was no significant difference between the Arborium Plus (300 mg/kg/d)-treated and atorvastation (5 mg/kg/d)-treated animal groups. Fur-

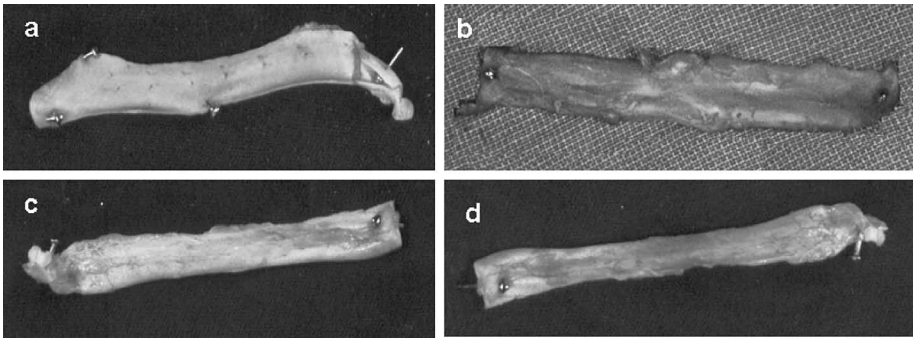

Fig. 7. Gross Morphological Changes of Thorasic Aortal Strips Stained with Oil Red O in Different Groups
(a) control animal aortal strip, (b) cholesterol fed rabbit aortal strip showing high lipid accumulation and thickened wall, (c) Arborium Plus and (d) Atorvastatin treated rabbit's thorasic aortal strip showing significant protection against lipid accumulation.

thermore, Oil Red O-stained rabbit aortal strips showed that gross morphologic differences were insignificant among the control, Arborium Plus and atorvastatin groups. However, red staining due to lipid accumulation in the endothelium of the high-fat diet-fed group clearly suggest the beneficial effects of Arborium Plus on hyperlipidemia.

Arborium Plus is rich in soluble fiber, which improves digestion and regulates elimination without causing laxative dependency, and rich in antioxidant polyphenolics, vitamins, tocopherol, and ascorbic acid.[25] Although the precise mechanism of action of Arborium Plus cannot be elucidated from this study, its ingredients may act on multiple pathways including cholesterol absorption and metabolism. Drugs like statins reduce LDL cholesterol by inhibiting HMG-CoA reductase in the liver. In our study, we observed similar effects of Aroborium Plus and a statin, suggesting the possible involvement of HMG-CoA reductase inhibition in the antiatherogenic action of this formulation.

Endothelial changes associated with inflammation, cell adhesion, recruitment, thrombosis, expression of regulatory cytokines, smooth muscle proliferation and apoptosis are major events contributing to coronary heart disease and atherosclerosis, where c-reactive protein plays a role in all of the events. The level of chronic inflammation is reflected by the level of CRP. In this regard, hs CRP-lowering effect of Arborium Plus is of intense scientific interest as an increase in hs CRP has been identified as a predictor of cardiovascular events.[26] Previous studies also showed that hs CRP acts as a mediator of disease progression by accentuating the inflammatory process ultimately leading to atherosclerosis or a cardiovascular event.[27–29] This suggests that Arborium Plus, like statins, prevented the atherogenic process by reducing inflammation in this study irrespective of the argument on whether hs CRP is a marker or mediator of cardiovascular risk. This also gives additional evidence for the multiple mechanisms of the antiatherogenic effect of Arborium Plus.

The present study provided experimental evidence of the antiatherogenic properties of Arborium Plus including preventing hyperlipidemia and inflammation. However, further studies are warranted to elucidate the precise mechanisms of action of this formulation, which would provide an understanding of pathologic processes and also strategies to prevent them.

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