Hypolipidemic Effect of Arborium Plus in Experimentally Induced Hypercholesteremic Rabbits

Devarakonda Murty,*† Enjamoori Rajesh,* Doonaboina Raghava,‡
Tangaraj Vijaya Raghavan,§ and Mukanthan Karupiah Munirajan Surulivel∥

*Shri Vishnu College of Pharmacy, Vizianagaram, West Godavari District, Andhra Pradesh, India,
†All India Institute of Medical Sciences, and §Department of Pharmacology Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Tamil Nadu, India

(Received October 25, 2009; Accepted January 12, 2010)

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 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 hypercholesterole-
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 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, heparin (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°C, 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. control; *** 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.

Changes in Serum Total Cholesterol

Serum total 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 (287.2±29.57, 357.3±10.46 vs. 1637±92.8 respectively) (Fig. 4). Arborium Plus and atorvastatin treatment prevented the increase in serum LDL cholesterol (p<0.001) as compared with the CHOL group (7.13±0.65, 8.96±0.42 vs. 9.15±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. 9.15±2.67).
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.

**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.\(^2\)\(^4\) 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 atorvastatin (5 mg/kg/d)-treated animal groups. Further...
thermore, Oil Red O-stained rabbit aortal strips showed that gross morphologic differences were insignif-
ificant among the control, Arborium Plus and ator-
vastatin groups. However, red staining due to lipid
accumulation in the endothelium of the high-fat diet-
fed group clearly suggest the beneficial effects of Ar-
borium Plus on hyperlipidemia.

Arborium Plus is rich in soluble fiber, which im-
proves 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 includ-
ing 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 Arborium Plus and a sta-
tin, suggesting the possible involvement of HMG-
CoA reductase inhibition in the antiatherogenic ac-
tion 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 coro-
nary heart disease and atherosclerosis, where c-reac-
tive 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 Arbo-
rium 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
This suggests that Arborium Plus, like sta-
tins, prevented the atherogenic process by reducing
inflammation in this study irrespective of the argu-
ment on whether hs CRP is a marker or mediator of
cardiovascular risk. This also gives additional evi-
dence for the multiple mechanisms of the antiathero-
genic effect of Arborium Plus.

The present study provided experimental evidence
of the antiatherogenic properties of Arborium Plus
including preventing hyperlipidemia and inflamma-
tion. However, further studies are warranted to eluci-
date the precise mechanisms of action of this formul-
atation, which would provide an understanding of
pathologic processes and also strategies to prevent

Acknowledgments The authors are grateful for
the financial support provided by Redhill Herbals
Pvt. Ltd., Dehradun.

REFERENCES
1) Selvaraj S., Ramasundaram S., Sundarama-
halingam M., Yakugaku Zasshi, 127, 385–388
(2007).
2) Gambhir D. S., Gambhir J. K., Sudha R., In-
(1999).
4) Ashakumary A., Vijayammal P. L., Indian J.
5) Gunde-Cimerman N., Int. J. Med. Mushrooms,
(1971).
7) Arinboor R., Venugopalan V. V., Sarinku-
mar K., Arumughan C., Sawhney R. C., J.
8) Guliyev V. B., Gul M., Yildirim A., J. Chro-
9) Geetha S., Sai R. M., Singh V., J. Ethnophar-
10) Gao X., Ohlander M., Jeppsson N., Björk L.,
Trajkovski V., J. Agric. Food Chem., 48,
11) Rösch D., Bergmann M., Knorr D., J. Agric.
13) Geetha S., Sai R. M., Mongia S. S., J.
14) Ganju L., Padwad Y., Singh R., Int. J. Im-
15) Agarwal S. S., Sharma K., Ind. J. Phar-
17) Rashid S., Uffelman K. D., Barrett P. H. R.,
18) Almuti K., Rimawi R., Spevack D., Int. J.