Influence of Abana on Experimental Atherogenesis in Hypercholesterolemic Rabbits

Ashok Kumar Tiwari and Jagdish D. Gode
Centre for Experimental Medicine and Surgery,
and
Govind Prasad Dubey,
Department of Basic Principles and Professor Incharge, Centre of Psychosomatic and Biofeedback Medicine,
Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

SUMMARY
An experimental, placebo-controlled study with rabbits was conducted to determine the role of Abana, an Ayurvedic preparation, on atherosclerosis. The results showed that Abana arrested the rise in TC, LDLc and VLDLc when administered along with an atherogenic diet for 60 days. In another phase of the study, when Abana was given from days 60-120 to rabbits who were made hyperlipidemic by feeding them an atherogenic diet for 60 days, Abana brought the TC, LDLc and VLDLc levels back to normal. On the other hand, the levels of the cardioprotective HDLc were elevated by Abana administration in both phases of the study.

Histological pictures showed pronounced reduction in the atherosclerosis involvement of the coronary artery following treatment with Abana. The architecture of the coronary artery returned almost to normal.

These findings substantiate the possible role of Abana in the reduction, prevention and reversal of atherosclerosis.

Key words: Hypercholesterolemia; Atherosclerosis; Abana

Many clinical trials to lower cholesterol levels in apparently healthy persons and in persons with known coronary heart disease CHD show a decrease in the incidence of nonfatal and fatal coronary events, respectively. It has also been shown that disturbances of lipid transport, which result from either an increased synthesis or a decreased breakdown of lipoproteins (which transport cholesterol and triglycerides through the plasma), may also cause one of the most important life-threatening diseases, atherosclerosis. Many coronary patients with normal or moderate levels of total cholesterol (TC) may have high levels of low-density lipoprotein cholesterol (LDLc) and low levels of high-density lipoprotein cholesterol (HDLc).

Studies have shown that aggressive management of hyperlipidemia in patients with established coronary disease significantly reduces the incidence of coronary events and mortality and promotes the regression of atherosclerotic plaques. High plasma triglyceride (TG) values are
associated with an increased risk for CHD, but some investigators question whether hypertriglyceridemia independently raises the risk. They suggest that instead, other lipid abnormalities, which are high by correlated with TG levels, are the true atherogenic factors. These include elevated levels of total cholesterol, LDLc and VLDLc and low levels of HDLc.

A drug which could reverse the above-mentioned changes would be highly beneficial in the prevention and treatment of ischemic heart disease. The present study evaluates the role of Abana, an Ayurvedic herbal preparation, on hyperlipidemia and atherosclerosis.

Abana is a safe, herbomineral preparation containing medicinal plants and mineral complexes reported to be useful in the therapy of cardiovascular diseases. The principal ingredients of Abana are *Terminalia arjuna, Withania somnifera, Tinospora cordifolia, Phyllanthus emblica, Boerhaavia diffusa, Centella asiatica, Convolvulus pluricaulis, Nardostachys jatamansi* and Shilajeet. Abana is prepared according to the principles of Ayurveda. The raw materials are used from the same source, collected in the proper season using the same part of the plant – all these help to impart uniform therapeutic activity. The proportions of the ingredients always remain constant and the uniformity of the final product is tested using thin layer chromatography and finger printing techniques.

**MATERIALS AND METHODS**

Twenty four adult male rabbits, weighing between 1.5 and 2 kg were kept for 15 days on rabbit pellets (Gold Mohur Rabbit Feed, Lipton India Ltd.) *ad libitum* in order to acclimatize them to laboratory conditions. After the period of acclimatization, the animals were divided into 4 groups and kept on an atherogenic diet (rich in 30% peanut oil and 5% cholesterol) for 60 days to induce hyperlipidemia and atherosclerosis. The experiment was designed as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Diet Day 0-60</th>
<th>Treatment Day 0-60</th>
<th>Diet Day 60-120</th>
<th>Treatment Day 60-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Atherogenic</td>
<td>Placebo</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Atherogenic</td>
<td>Abana 500 mg/kg daily</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Atherogenic</td>
<td>Placebo</td>
<td>Normal rabbit feed</td>
<td>Placebo</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Atherogenic</td>
<td>Placebo</td>
<td>Normal rabbit feed</td>
<td>Abana 500 mg/kg daily</td>
</tr>
</tbody>
</table>

**Blood lipid analysis**: Blood samples were collected in plain vials. Estimations were carried out for serum cholesterol, HDL cholesterol and triglycerides after 12 to 24 hours of fasting. Values of atherogenic lipoprotein cholesterol (VLDLc and LDLc) were calculated by subtracting the value of HDLc from total cholesterol.
**Morphological and histological evaluation of atherosclerosis:** The rabbits were sacrificed at the end of the experimental period. Their hearts were removed, washed and kept in 10% formalin solution and processed for light microscopic sections.

The aortas were removed intact from the aortic arch to the iliac bifurcation after being perfused *in situ* with 0.2M phosphate buffered saline containing papaverine (0.12 mg/ml) to avoid vasoconstriction. As the adventitial debris was stripped, the aortas were cut open longitudinally through a dorsal incision and were fixed in 10% formalin. The fixed aortas were stained with Sudan IV to reveal sudanophilic plaques. After staining they were pinned open and flattened. A template was then made by tracing the outlines of the aorta and the atheromatous lesions on a clear plastic sheet. Each template was magnified (x2) and photographed. Morphometric assessment of the percentage of the total aorta covered with lipid deposits (Sudan-positive area) was done by planimetry.

The animals were closely followed during the experimental period. Their food intake was recorded daily and each animal was weighed bi-monthly to detect any change.

**RESULTS**

An atherogenic diet produces a marked increase in body weight, serum cholesterol and other lipoproteins such as LDLc and VLDLc and a fall in HDLc. During the 60 days of treatment with an atherogenic diet, there was a significant increase in mean body weight in both groups. But on withdrawal of the atherogenic diet, there was reduction in body weight in both groups after 60-120 days (Table 1).

| Table 1: Body weight changes (in kg) in established hyperlipidemic rabbits, and 60 days after return to normal diet, without and with Abana treatment vs Control |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Initial weight | Atherogenic diet (0-60 days) | Normal diet (60-120 days) |
| Placebo (Group I) | Abana (Group II) | Placebo (Group III) | Abana (Group IV) |
| 1.78 | 2.08* | 2.78* | 1.79 | 2.12 |
| *p<0.05 as compared to initial value. |

Data in Table 2 show greater than ten-fold increases in TC, LDLc + VLDLc and triglycerides in Group I (control) animals fed an atherogenic diet for 60 days, while the cardioprotective HDLc showed a lower value (Table 2, Group I).

Administration of Abana (500 mg/kg) along with an atherogenic diet for 60 days arrested the rise in TC, LDLc + VLDLc and triglycerides to a considerable extent. The fall in HDLc was prevented in the Abana-treated group (Table 2, Group II).

Those animals kept on an atherogenic diet only and then switched to normal rabbit feed after 60 days, showed a gradual decline in the levels of these parameters. The levels were significantly higher even after 120 days on a normal diet (Table 3, Group III).
In Group IV animals, kept on an atherogenic diet for 60 days and then on Abana (500 mg/kg) for another 60 days, there was a rapid fall, and after 120 days the levels of TC, LDLc + VLDLc and TG were restored to normal (Table 3, Group IV).

### Table 2: Lipid and lipoprotein values in rabbits receiving Abana along with Atherogenic diet vs controls, values in mg/dl

<table>
<thead>
<tr>
<th></th>
<th>Group I – Control</th>
<th>Group II – Abana-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>After 60 days</td>
</tr>
<tr>
<td>TC</td>
<td>59.83 ± 7.11</td>
<td>580.33 ± 164.94*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>28.33 ± 8.16</td>
<td>20.00 ± 6.32</td>
</tr>
<tr>
<td>LDL-c + VLDL-c</td>
<td>31.50 ± 8.80</td>
<td>460.83 ± 163.29*</td>
</tr>
<tr>
<td>TG</td>
<td>20.83 ± 5.85</td>
<td>500.00 ± 176.07*</td>
</tr>
</tbody>
</table>

*\(p<0.001\), as compared to basal reading.

**\(p<0.001\), as compared to basal reading and Group I.

The extent of atherosclerotic involvement of the aorta was significantly less under the influence of Abana (Table 4). The atherogenic diet resulted in the development of atheromatous plaques in the aorta involving 40% of the areas; withdrawing the diet did not cause much regression [Groups I and III (Controls)]. On the other hand, treatment with Abana and an atherogenic diet, or after changeover to normal diet + Abana, caused a marked reduction in the atheromatous area [Groups II and IV (both Abana-treated)].

The above findings were very well substantiated by the histological pictures which showed blockage of the arterial lumen with massive lipid deposits under the influence of a hyperlipidemic diet (Fig. 2). There were signs of reversal of atherosclerotic plaques following switchover of the diet from a hyperlipidemic one to normal pellets after 60 days (Figs. 3 and 4). Fig 5 shows a pronounced reduction the atherosclerotic involvement of the coronary artery following treatment with Abana. The architecture of the coronary artery almost returned to normal (Fig 1).
Table 4: Aortic atherosclerotic involvement (% ± SD) of the aortic surface covering fatty streaks

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>40.50% ± 5.39</td>
</tr>
<tr>
<td>Group II (Abana-treated from Day 0-60)</td>
<td>24.17% ± 4.22*</td>
</tr>
<tr>
<td>Group III (Control)</td>
<td>31.17% ± 3.76</td>
</tr>
<tr>
<td>Group IV (Abana-treated from Day 60-120)</td>
<td>18.67% ± 3.44**</td>
</tr>
</tbody>
</table>

*p<0.05, as compared to Group I
*p<0.05, as compared to Group III.

DISCUSSION
It is well known that lowering the serum cholesterol level reduces the risk of coronary heart disease. Many clinical studies correlated a strong association between serum cholesterol levels and coronary artery disease. Since elevation of LDLc and VLDLc increases coronary risks, efforts should be made to reduce the concentrations of these lipoproteins.

Abana is a complex herbal preparation with important plant ingredients such as *Terminalia arjuna*, *Withania somnifera* and *Boerhaavia diffusa*, known for their beneficial effects in
cardiovascular diseases, as reported in the Indian System of Medicine (Ayurveda). *Terminalia arjuna* has been reported to possess a hypolipidemic effect in experimental models\(^{11}\). Ayurveda describes many combinations of ingredients in different proportions so formulated that the end product confers optimal therapeutic benefits.

There is strong evidence from several studies that the extent of reduction in the incidence of CHD is directly related to the magnitude of reduction in LDLc and VLDLc levels\(^{12}\).

Studies have also shown that atherosclerotic lesions can be retarded, prevented or reversed in experimental models and that healing of the damaged arterial endothelium can be achieved by substantial lowering of blood lipids after a relatively short period on low-lipid diet or with drugs\(^{13,14}\).

Our study showed that treatment with Abana not only checked the rise in total serum cholesterol and other atherogenic lipoproteins (LDL and VLDL cholesterol), when administered along with an atherogenic diet, but also helped in reducing their levels significantly. These findings are in agreement with the fact that a drug which can decrease the levels of cholesterol and other atherogenic lipoproteins will prove highly beneficial in the prevention and reversal of atherosclerosis\(^2\).

**REFERENCES**


