Antihypercholesterolemic Activity of Garlicky Principles of Adenocalymma Alliaceum

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The volatile oil of Adenocalymma alliaceum (Bignoniaceae) composed of Diallyl di-, tri- , and tetrasulphides (5:5:1) showed antihypercholesterolemic activity comparable to that of clofibrate in diet-induced hypercholesterolemic rats.

Adenocalymma alliaceum mart (Bignoniaceae) is a beautiful ornamental climber native to Brazil, but grown as a garden plant in many tropical and subtropical countries. A garlicky smell is emitted by all parts of the plant on crushing. Our chemical investigation of leaves and flowers of the plant resulted in the isolation of a volatile oil and flavonoids. Using GLC-MS analysis we have established that the volatile oil from the flowers consists entirely of diallyl di-, tri- and tetrasulphides while the oil from the leaves contains in addition 1-octen-3-ol. The presence of allylic sulphides was formerly regarded as a specific characteristic of garlic (Allium sativum) and a few closely related Allium species, but never before encountered within the class of dicotyledonous angiosperms. However, garlic oil is a veritable mixture of several components which include dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide, diallyl sulphide, diallyl disulphide and diallyl trisulphide.

Garlic is known to have many therapeutic uses among which, its use in the treatment of cardiac ailments, particularly hypercholesterolemia, has received special attention, although the precise chemical composition of the preparations tested was not ascertained. Since we have obtained pure volatile oil consisting of diallyl di-tri- and tetrasulphides (5:5:1) from the flowers of A. alliaceum we have studied its antihypercholesterolemic activity in comparison with a standard antilipemic agent, clofibrate, and the results recorded herein.

EXPERIMENTAL

The volatile oil used in this work was isolated by steam distillation of flowers of A. alliaceum collected in the Andhra University campus. The experimental procedure for determining the antihypercholesterolemic activity is similar to that reported for curcumin.

In a typical experiment, twelve male albino rats each weighing about 50 g were distributed in four groups of three rats in each group. The animals in all groups were given on a basal diet (Table 1). Before the start of the experiment all the animals were given the basal diet for 2 days. There after rats in group I (control) were kept on basal diet and the groups II, III and IV on hypercholesterolemic diet throughout the experimental period. The hypercholesterolemic diet contained 65.5 parts of starch, 1 part of cholesterol and 0.5 parts of sodium tauroglycocholate instead of 67 parts of starch in the basal diet. The remaining constituents of hypercholesterolemic diet were same as that of basal diet. Rats in group III were given in addition a daily oral dose of 40mg/kg of volatile oil (in coconut oil) from A. alliaceum while those in group IV a daily oral dose of 40 mg/kg of clofibrate (in coconut oil).
Table I: Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Parts per 100 parts of the diet</th>
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<tbody>
<tr>
<td>Starch</td>
<td>67</td>
</tr>
<tr>
<td>casein</td>
<td>10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
</tr>
<tr>
<td>Fat (hydrogenated groundnut oil)</td>
<td>10</td>
</tr>
<tr>
<td>Salt mixture (limestone 1% with minerals)</td>
<td>2</td>
</tr>
<tr>
<td>Vitaminised starch</td>
<td>1</td>
</tr>
</tbody>
</table>

All the three rats of each group were placed in a single cage. Fresh food appropriate to each group and water were given ad libitum. During the experimental period the food consumed and weight gained were recorded every 24 hours. During the last three days of the experiment feces were collected every morning, dried and preserved for analysis.

At the end of nine days the animals were starved for 24 hours and anaesthetised by intraperitoneal injection of 5% sodium pentobarbital solution. Blood was withdrawn from the aorta. Plasma was separated by centrifuging the blood. Livers were excised, blotted with filter paper to remove the adhering blood. Both livers and plasma were stored in deep freeze until analysed.

Total cholesterol in plasma was estimated according to Zlatkis method\textsuperscript{12} and plasma glycerides by chromotropic acid method.\textsuperscript{13}

The excised livers were separated according to the appropriate group and the liver (1.0 g) was homogenised with 0.9% saline (1 ml). The homogenate was extracted with acetone-ethanol mixture (1:1) warmed to 60\textdegree, cooled and the filtrate and washings made to volume. Total cholesterol in the liver was estimated on aliquots.

Feces were pooled according to individual groups and the adhering hair and food particles were removed with the aid of forceps and thoroughly ground in a mortar. Fecal bile acids were estimated according to the procedure of Mosbach et al.\textsuperscript{14} by measuring the absorption at 320 nm for cholic acid and 385 nm for deoxy cholic acid.

**RESULTS AND DISCUSSION**

Table 2 shows the results of the weight gain during the experimental period, the levels of plasma and liver cholesterol, plasma triglycerides and fecal bile acids.

The gain in weight for the rats maintained on hypercholesterolemic diet (Group II) was more than the rest (Groups I, III and IV). The increase in weight in rats administered with volatile oil of *A. alliaceum* or clofibrate was less when compared to rats fed with basal diet. Mild diarrhoea was observed on the first two days in the rats of group III and IV.

From the results it was observed that in the rats administered with the volatile oil *A. alliaceum* (Group III) plasma cholesterol level was reduced to an extent of 20.5% when compared to that of hypercholesterolemic group (Group II), whereas in clofibrate administered rats (Group IV), the reduction was 30.7%.

Liver cholesterol increased to an extent of 22.8% in cholesterol fed rats compared to rats fed with basal diet whereas this decreased to an extent of 15.5% in volatile oil administered rats and 17.5% in clofibrate administered ones.

Plasma triglycerides increased to a significant extent in the hypercholesterolemic rats compared to rats fed with basal diet. In the rats given volatile oil triglycerides decreased to an extent of 23.9% compared to hypercholesterolemic group. In the clofibrate fed rats the triglycerides level decreased by about 37.9%.
Table 2: Weight gain, levels of plasma and liver cholesterol, plasma triglycerides and fecal bile acids in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt. gain in 9 days g±SEM</th>
<th>Total cholesterol</th>
<th>Plasma triglycerides mg/100 ml ± SEM</th>
<th>Excreted bile acids mg/rat/day ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (mg/100 ml ± SEM)</td>
<td>Liver mg/g ± SEM</td>
<td></td>
</tr>
<tr>
<td>Group I (Control)</td>
<td>2.1 ± 0.385</td>
<td>78.66 ± 1.334</td>
<td>6.46 ± 0.33</td>
<td>68.3 ± 1.710</td>
</tr>
<tr>
<td>Group II</td>
<td>3.66 ± 0.258</td>
<td>117.33 ± 3.335</td>
<td>7.93 ± 0.33</td>
<td>96.6 ± 3.309</td>
</tr>
<tr>
<td>Group III</td>
<td>1.66 ± 0.255</td>
<td>90.33 ± 2.335</td>
<td>6.73 ± 0.26</td>
<td>73.3 ± 1.720</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.40 ± 0.385</td>
<td>81.23 ± 2.316</td>
<td>6.53 ± 0.33</td>
<td>60.2 ± 2.685</td>
</tr>
</tbody>
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SEM: Standard error of the mean

Estimation of the fecal bile acids showed that in the volatile oil administered rats there was an increased excretion of bile acids to an extent of about 25% compared to rats fed on basal diet and to about 23% compared to rats fed on hypercholesterolemic diet. A decrease in fecal bile acid excretion was observed with clofibrate when compared to rats fed on basal diet.

The present study shows that the volatile oil of A. alliaceum possesses antihypercholesterolemic activity comparable to that of clofibrate and the activity is attributable to the diallyl sulphides present in the volatile oil. The exact mechanism by which the volatile principles reduced the plasma cholesterol is not clear but the observation of diminished tissue lipids and increased excretion of bile acids (cholic acid and deoxycholic acid, the end products of cholesterol metabolism) suggest that it may act both by affecting endogenous synthesis of cholesterol in the liver like clofibrate and by increasing excretion of cholesterol end products. The currently known mechanism of action of clofibrate is that it produces a change in the plasma lipid profile by increasing the activity of lipoprotein lipase and also by increasing the hepatic clearance of VLDL and probably LDL.\textsuperscript{15,16}

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REFERENCES


