Evaluation of Anti-Hypercholesterolemic Activity of *Ammomum subulatum* Seeds Extract

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ABSTRACT

*Ammomum subulatum* is a perennial plant cultivated in swampy places in Bengal, Sikkim, Assam and Tamil Nadu. Plant bears fruit having numerous seeds which are traditionally used in spice. Fruits are used as stimulant, aromatic, stomachic, aphrodisiac, in infection of teeth and gums. The present study is an attempt to explore the Anti-Hypercholesterolemic effect of acetone and methanolic extract of seeds of *Ammomum subulatum*, using experimental model: Triton WR 1339 induced hypercholesterolemia in rats. Dose of 200 mg/ml, 400mg/ml and 800 mg/ml of acetone and methanolic extract were evaluated for their Anti-Hypercholesterolemic activity against triton WR 1339 induced hypercholesterolemia in rat. Both the extracts were able to show Anti-Hypercholesterolemic activity in dose dependant manner as compared with standard drug Atorvastatin 5 mg/kg. The data were found statistically significant by using one way ANOVA (P< 0.001).our data suggest that Ammomum subulatum significantly reduced Serum total cholesterol, triglyceride, LDL and increase HDL, VLDL in the triton rats treated with drugs for 28 days.

Keywords: Ammomum subulatum; Zingiberaceae; anti-hypercholesterolemia; triton WR 1339, Atorvastatin, cholesterol, triglyceride, HDL, VLDL, total lipid

Introduction

*Ammomum subulatum* Roxb. (Family: Zingiberaceae) commonly known as Large or Greater Cardamom, Moti elaichi. Large cardamom is a tall perennial herb found in Eastern Himalayas and sub-Himalayan region of West Bengal, Assam and Sikkim. The seeds are aromatic pungent, stimulant, stomachic, alexipharmic and astringent. Traditionally, it is used to treat stomach pain, flatulence, belching, indigestion vomiting, malarial disorders, and drunkenness from alcohol Consumption. In Ayurvedic and Unani medicines large cardamoms are used as a preventive as well as a curative for throat troubles, congestion of lungs.

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inflammation of eyelids, digestive disorders and in the treatment of pulmonary tuberculosis. A. subulatum contains 1, 8-Cineole, α-pinene and β-pinene and geraniol, subulin, chalcone, cardamonin and a flavanone, alpinetin.1-5

Material and Methods

Plant Material and Extraction
The fruits of Amomum subulatum Roxb were collected from local market of Modasa and authenticated by Dr. H. B. Singh Scientist and Head of Raw Materials Herbarium & Museum Dept of National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR) and preserved the herbarium in Smt. R. B. Patel Mahila Pharmacy College, Atkot, Rajkot, Gujarat. The seeds were dried at room temperature and mechanically powdered to obtain a coarse powder; defatted with petroleum ether (60-80°C) and Soxhlet extracted with acetone and methanol. Solvent removal under reduced pressure was afforded by acetone and methanolic extract. The dry methanolic extract was stored in cool and dry place which was further used for evaluation of the hypercholesterolemic activity.

Animals
Adult male Wistar rats weighing around 180-200g were obtained from zydus healthcare, Ahmedabad, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±20C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines. The study protocol was approved by institutional animal ethical commit, RBPMPC, Atkot, India.

Animal described as fasted were deprived of food for 16 h but free access to water.

Experimental design
Adult wistar rat with an initial body weight of 180 to 200g were taken, and divided into eleven groups each containing six animals.

Group I: Normal control (CON): They were administered with vehicle (saline) for 28 days. They were fed with standard laboratory diet and water ad libitum. Triton was not given.

Group II: Disease control (TRT): They were administered with vehicle (saline) for 28 days. They were fed with standard laboratory diet and 1 ml of 10% triton in saline.

Group III: Acetone extract 800 mg/kg alone (ACE800+CON): Acetone extract of A. subulatum seeds (800 mg/kg, p.o.), was administered for 28 days. Triton was not given.

Group IV: Methanol extract 800 mg/kg alone (ME800+CON): Methanol extract of A. subulatum seeds (800 mg/kg, p.o.), was administered for 28 days. Triton was not given.

Group V: Acetone extract 200mg/kg + Triton WR 1339(ACE200+TRT): Acetone extract of A. subulatum seeds (200 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.
Group VI: Acetone extract 400 mg/kg + Triton WR 1339 (ACE400+TRT): Acetone extract of *A. subulatum seeds* (400 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.

Group VII: Acetone extract 800mg/kg + Triton WR 1339 (ACE800+TRT): Acetone extract of *A. subulatum seeds* (800 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.

Group VIII: Methanol extract 200mg/kg + Triton WR 1339 (ME200+TRT): Methanol extract of *A. subulatum seeds* (200 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.

Group IX: Methanol extract 400mg/kg + Triton WR 1339 (ME400+TRT): Methanol extract of *A. subulatum seeds* (400 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.

Group X: Methanol extract 800mg/kg + Triton WR 1339 (ME800+TRT): Methanol extract of *A. subulatum seeds* (800 mg/kg, p.o.), was administered for 28 days and on 28th day 1ml of 10% triton in saline was given.

Group XI: Atorvastatin 5 mg/kg + Triton WR 1339 (ATR 5 + TRT): Atorvastatin (5 mg/kg/day, p.o) was administered for 28 days and on 28th day 1 ml of 10% triton in saline was given.

After 24 hours of triton injection or drug treatment, collection of blood was done and biochemical estimations of the parameters were carried out.

Biochemical parameter:
Collection of serum:

The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4°C until used.

Estimation of parameters:
The serum so collected was used for estimation of serum cholesterol, serum triglyceride, serum HDL, serum LDL. Triglycerides were estimated by GPO Method\(^7\), Cholesterol and high density lipoprotein (HDL) by CHO /POD Phosphotungstate Method\(^8,9\), and low density lipoprotein (LDL) and very low density lipoprotein concentrations were determined by Friedewald formula.\(^8,10\)

Statistical analysis:

All the values are expressed as mean S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graphpad.). Differences were considered to be statistically significant when \( p < 0.05 \).
Result and Discussion

Table 1: Effects of acetone and methanol extract of *Amomum subulatum* Roxb. on lipid profile in normal rats and triton (1 ml of 10% in saline) induced hypercholesterolemia in rats (*p<0.05, **p<0.01, ***p<0.001, NS p>0.05)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Disease</th>
<th>ACE alone</th>
<th>ME alone</th>
<th>AC E 200</th>
<th>AC E 400</th>
<th>AC E 800</th>
<th>ME 200</th>
<th>ME 400</th>
<th>ME 800</th>
<th>ATO RV</th>
</tr>
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<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>80.67 ± 2.716</td>
<td>250.3 ± 1.542</td>
<td>80.67 ± 1.542</td>
<td>79.6 ± 1.145</td>
<td>152.7 ± 3.3</td>
<td>126.3 ± 2.40</td>
<td>98.8 ± 2.27</td>
<td>161 ± 3.55</td>
<td>130 ± 2.13</td>
<td>93.5 ± 2.06</td>
<td>90.17 ± 1.74</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>64.50 ± 3.449</td>
<td>117.2 ± 3.106</td>
<td>65.83 ± 2.871</td>
<td>66.3 ± 3.624</td>
<td>100.3 ± 2.750</td>
<td>95.0 ± 3.631</td>
<td>90.8 ± 3.097</td>
<td>97.5 ± 3.291</td>
<td>92.6 ± 3.157</td>
<td>87.3 ± 3.152</td>
<td>80.00 ± 4.094</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>41.33 ± 2.949</td>
<td>12.17 ± 0.833</td>
<td>42.83 ± 2.949</td>
<td>38.8 ± 2.156</td>
<td>28.1 ± 1.502</td>
<td>27.5 ± 4.342</td>
<td>31.6 ± 1.567</td>
<td>28.5 ± 2.150</td>
<td>30.6 ± 3.117</td>
<td>33.5 ± 4.370</td>
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<td>VLDL (mg/dl)</td>
<td>8.267 ± 0.589</td>
<td>2.333 ± 0.166</td>
<td>8.567 ± 0.166</td>
<td>7.76 ± 0.603</td>
<td>5.63 ± 0.433</td>
<td>5.50 ± 0.868</td>
<td>6.33 ± 0.868</td>
<td>5.70 ± 0.318</td>
<td>6.13 ± 0.433</td>
<td>6.70 ± 0.621</td>
<td>7.033 ± 0.8739</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>31.07 ± 2.291</td>
<td>235.7 ± 3.323</td>
<td>29.27 ± 2.491</td>
<td>33.0 ± 2.583</td>
<td>118.3 ± 3.641</td>
<td>93.3 ± 3.199</td>
<td>60.8 ± 4.243</td>
<td>126.3 ± 4.668</td>
<td>94.0 ± 3.752</td>
<td>53.3 ± 4.842</td>
<td>47.97 ± 3.597</td>
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<tr>
<td>HDL ratio</td>
<td>107.7 ± 13.91</td>
<td>5.130 ± 1.954</td>
<td>116.6 ± 13.91</td>
<td>98.0 ± 12.39</td>
<td>22.9 ± 2.282</td>
<td>28.2 ± 3.861</td>
<td>49.2 ± 8.867</td>
<td>21.7 ± 1.837</td>
<td>31.0 ± 2.968</td>
<td>58.9 ± 9.107</td>
<td>66.62 ± 10.93</td>
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<td>Atherogenic index</td>
<td>1.592 ± 0.830</td>
<td>9.891 ± 0.169</td>
<td>1.587 ± 0.169</td>
<td>1.73 ± 0.065</td>
<td>3.68 ± 0.300</td>
<td>3.68 ± 0.430</td>
<td>3.18 ± 0.472</td>
<td>3.45 ± 0.152</td>
<td>3.09 ± 0.217</td>
<td>2.71 ± 0.264</td>
<td>2.472 ± 0.343</td>
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**Figure 1:** Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) and Atorvastatin (5mg/kg) on Serum total cholesterol and triglycerides in normal rats and triton (1 ml of 10% in saline) induced hypercholesterolemia in rats (* p<0.05, **p<0.01, ***p<0.001)

**Figure 2:** Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) and Atorvastatin (5mg/kg) on Serum HDL cholesterol and LDL cholesterol in normal rats and triton (1 ml of 10% in saline) induced hypercholesterolemia in rats (* p<0.05, **p<0.01, ***p<0.001)
**Figure 3:** Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) and Atorvastatin (5mg/kg) on Serum VLDL cholesterol and HDL ratio in normal rats and triton (1 ml of 10% in saline) induced hypercholesterolemia in rats (* p<0.05, ** p<0.01, *** p<0.001)

**Figure 4:** Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) and Atorvastatin (5mg/kg) on atherogenic index in normal rats and triton (1 ml of 10% in saline) induced hypercholesterolemia in rats (* p<0.05, ** p<0.01, *** p<0.001)
There was significant (P<0.001) increase in serum total cholesterol of triton treated animals as compared to the normal animals. Both extracts of Amomum subulatum (200 mg/kg, 400 mg/kg and 800 mg/kg) significantly (P<0.001) reduced the TC level as compared to the triton treated group.

In case of HDL cholesterol, Both extracts of Amomum subulatum increased the HDL levels significantly (p<0.001) as compared to triton treated group at all the dose levels. Amomum subulatum reduce the serum LDL levels as compared to triton alone treated animals. Both extracts of Amomum subulatum significantly (p<0.001 in all the cases) increased the serum VLDL levels at the dose level of 200, 400 and 800 mg/kg.

Conclusions:
The quality of life of millions of people is adversely affected by angina and heart failure caused by coronary artery disease (CAD), by intermittent claudication secondary to peripheral vascular disease and by transient ischemic episodes secondary to cerebrovascular disease. It is for these reasons so much attention is directed toward understanding the etiology of hyperlipidemia and the development of effective therapeutic strategies. Hyperlipidemia has been defined in the past as plasma cholesterol and triglyceride levels that exceed “normal” levels. Diets and drug therapy for hypercholesterolemia is clearly indicated for individuals with existing CAD, as well as for individuals with multiple risk factors, and in particular, those with a strong family history of cardiovascular disease and those with diabetes. There are certain types of extreme hypertriglyceridemia that can cause potentially life-threatening pancreatitis and therapy to lower triglyceride levels in these patients will prove beneficial.

Lipid powering is the balance between reducing the risks of events (including death) related to coronary artery disease and the risk of drug treatment. Quite apart from the risk of drug toxicity, lowering lipid concentrations might in itself, however achieved, increase the risk in other ways.

Lowering cholesterol concentrations may alter cellular function and behaviour and this warrants further investigations. One view would be that when the benefit of therapy is enhanced, as it is in those at very high risk in secondary prevention, the possible increase in risk of non-coronary artery disease can be accepted.

It is generally agreed that diet and weight control are the first line treatment of CAD patients with high cholesterol or triglyceride blood levels. Such measures are continued for several months and in many patients, this is sufficient to reduce the concentration of cholesterol or triglycerides. However, there are patients who do not respond adequately to non-drug management and in such cases, the use of lipid lowering agent is usually related to the dominant abnormality (elevated plasma concentration of cholesterol or triglyceride, or both). On occasion, combination treatment with reduced doses of two drugs is more effective than a single agent with fewer side effects. In spite of the availability of
various antihyperlipidemic agents, there is increase in coronary heart diseases and risk of congestive heart failure. Thus there is still considerable interest in synthesis and evaluation of new antihyperlipidemic agents. So also, research has been carried out relevant to herbal indigenous drugs, which does not lower cholesterol levels but also prevents further disaster caused by oxidized form of lipids by acting as a free radical scavenger.

Increase in the serum lipid profile with triton treatment also was found to be significantly decreasing with *Amomum subulatum* and Atorvastatin treatment.

**References**